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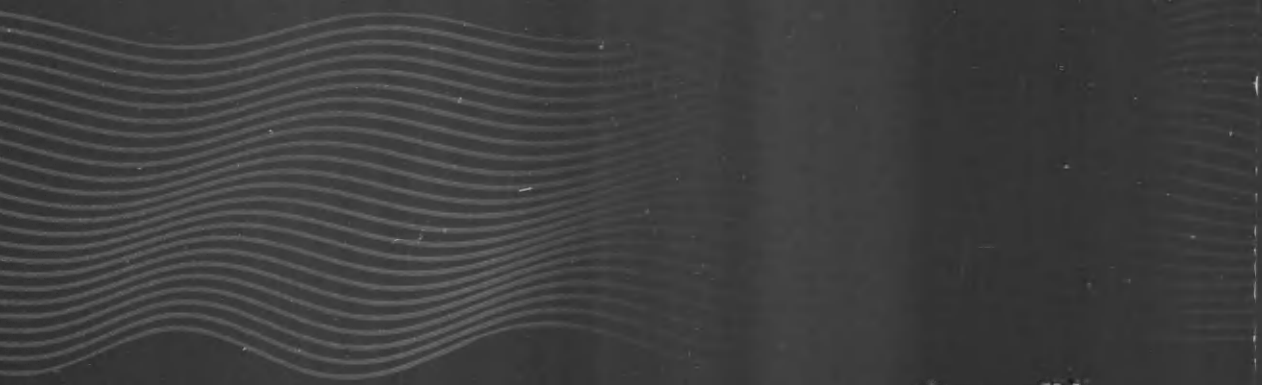

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Performance of Domestic, Hybrid, and Wild Strains of Brook Trout,[1] *Salvelinus fontinalis*, after Stocking: the Impact of Intra- and Interspecific Competition

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Lachance, S., and P. Magnan. 1990. Performance of domestic, hybrid, and wild strains of brook trout, *Salvelinus fontinalis*, after stocking: the impact of intra- and interspecific competition. Can. J. Fish. Aquat. Sci. 47: 2278–2284.

Wild and hybrid strains of brook trout, *Salvelinus fontinalis*, showed better rates of recovery (by angling) and yield (kilograms of fish recovered per kilogram planted) than a domestic strain, during the 2 yr following planting, in six small oligotrophic lakes of the Laurentian Shield. Native brook trout and white sucker, *Catostomus commersoni*, had a significant impact on planted brook trout. Recovery, percent of increment in weight and yield of each planted strain were inversely correlated with (1) the relative abundance of native brook trout, and (2) the occurrence of white sucker, supporting hypotheses of intra- and interspecific competition. Furthermore, the response variables were also inversely correlated with the number of potential competitors, indicating that the impact of native brook trout and white sucker was additive. The recovery in number of planted trout (both years and all strains) was approximately four times higher in lakes with effectively no competitors than in lakes containing both white sucker and native brook trout; the increase in weight was nearly three times higher, and the yield was more than nine times higher. The performances of planted fish were intermediate in the lake containing only native brook trout as competitor.

Une lignée sauvage et une lignée hybride d'omble de fontaine, *Salvelinus fontinalis*, ont montré des taux de recapture (par la pêche sportive) et des rendements (kilogrammes de poissons recapturés par kilogramme de poissons ensemencés) supérieurs à ceux d'une lignée domestique, 2 ans après leur ensemencement dans six petits lacs oligotrophes du bouclier Laurentien. Les ombles de fontaine indigènes et les meuniers noirs, *Catostomus commersoni*, avaient un impact significatif sur les ombles de fontaine ensemencés. Le taux de recapture, le pourcentage d'augmentation en poids et le rendement de chaque lignée ensemencée étaient inversement corrélés (1) à l'abondance relative des ombles de fontaine indigènes et (2) à la présence du meunier noir, supportant les hypothèses de compétition intra- et interspécifique. De plus, les variables mesurées étaient également corrélées de façon inverse au nombre de compétiteurs potentiels, indiquant que l'impact des ombles de fontaine indigènes et des meuniers noirs était additif. Le taux de recapture des ombles ensemencés (pour les deux années et toutes les lignées) était approximativement quatre fois plus élevé dans les lacs ne contenant effectivement pas de compétiteurs que dans les lacs contenant du meunier noir et de l'omble de fontaine indigène; l'augmentation en poids était près de trois fois plus élevée et le rendement était plus de neuf fois plus élevé. Les performances des poissons ensemencés étaient intermédiaires dans les lacs contenant seulement de l'omble de fontaine indigène comme compétiteur.

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Many works have documented the better performance of hybrid and wild strains of brook trout, *Salvelinus fontinalis*, over domestic strains after stocking (Flick and Webster 1964, 1976; Fraser 1972, 1978, 1980, 1981; Keller and Plosila 1981; Webster and Flick 1981). The recovery, survival, and yield of wild and hybrid strains were generally higher than those of domestic strains in natural and semi-natural environments. However, although brook trout is often found with other fish species in the Precambrian Shield lakes (Fraser 1981; Magnan 1988, 1989), no study has systematically investigated the impact of interspecific competition on the success of different strains of trout. An exception is the work of Fraser (1981), but interpretation of his work is complicated because brook trout were planted in lakes containing multispecies communities. Furthermore, no study has investigated the impact of intraspecific competition between native and planted brook trout after stocking. It is known that many lakes which contain native salmonids must be stocked to compensate for poor recruitment (Wydoski 1986). More specific information of this kind would thus be of prime importance in maximizing the success of different stocking programs in eastern North America.

The goals of the present study were (1) to compare the recovery, growth, and yield of three strains of brook trout (domestic, hybrid, and wild) in six small oligotrophic lakes of the Laurentian Shield, and (2) to investigate the impact of intra- and interspecific competition on these strains, using a "field experiment" approach (e.g. Diamond 1986). The strains were stocked at an equal rate in each lake, all containing various densities of native brook trout and three also containing white

[1]The authors prefer the common name "charr" to designate species of the genus *Salvelinus*.

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sucker, *Catostomus commersoni*. The white sucker was selected as the competitor because it frequently occurs with brook trout (Scott and Crossman 1973; Fraser 1981; Magnan 1988, 1989), and also because it competes with trout for food which is reflected in trout production, spatial distribution, feeding habits and morphology (Magnan 1988; S. Tremblay and P. Magnan, unpubl. data).

Materials and Methods

Origin of the Different Strains

Domestic and hybrid strains were reared at the Lake des Ecorces hatchery, ministère du Loisir, de la Chasse et de la Pêche du Québec (MLCP), which is located near Mont-Laurier (Québec). The domestic strain originated from a New Hampshire stock imported by the MLCP in 1981 and hatchery-reared for at least 50 yr. This strain, called NASH by the MLCP, corresponds to the "Cortland" domestic strain used by Webster and Flick (1981), and propagated by the United States Fish and Wildlife Service. The hybrid strain originated from a cross between domestic females (NASH) and wild males from the Sainte-Marguerite river, located in the Saguenay region (Québec). The wild strain was reared at the Baldwin hatchery (MLCP) near Sherbrooke (Québec), and was the F_1 generation of parents originating from Lake Banville, in Des Laurentides Reserve, near Québec City. Individuals of the three strains were reared in similar conditions from the egg to the fall fingerling stage.

Prior to planting, the individuals of the three strains were marked (fin clipping) to allow subsequent identification in the field. The choice of fins to be clipped was based on Mears (1976) to avoid regeneration and locomotion problems. The adipose fin was excised from the wild strain, right pelvic fin from domestic strain, and left pelvic fin from the hybrid strain. Prior to marking, the fish were lightly anaesthetized with MS-222 or 3-methyl-pentanol. Fish were marked on September 26 and 27, 1985, 10–13 d before planting. Mortality due to marking stress was negligible. Following marking, 117 domestic, 118 hybrid and 109 wild trout were sampled at random, weighed (± 0.01 g) and measured (± 1 mm TL).

Study Lakes

The six lakes studied were located in the Mastigouche Reserve, north of Trois-Rivières (Québec) (46°40′N, 73°20′W) (Table 1). The lakes of this region are typical of small oligotrophic temperate zone lakes with respect to area, mean depth, conductivity, dissolved oxygen, thermal stratification, and Secchi disk transparency (see Magnan 1982, 1988). As the study lakes were located within a 325 km² radius, they were exposed to similar climatic conditions.

The lakes were divided into two groups: (1) those containing native brook trout with or without northern redbelly dace, *Phoxinus eos*, called "brook trout lakes," and (2) those containing native brook trout, white sucker and northern redbelly dace, "brook trout and white sucker lakes" (Table 1). Almost all lakes contained northern redbelly dace but their presence did not significantly affect the mean yield of brook trout in the exploited lakes of the Mastigouche Reserve (P. Magnan, unpubl. data). Lake Grignon contained pearl dace, *Semotilus margarita*, but gillnet fishing indicated they represented less than 1% of the total fish biomass. Although not quantified, the same was probably true for northern redbelly dace. Thus our classification into two types of lake is representative of the species present for the purpose of this study. Native trout from Lake Brochard (brook trout lake) and from lakes Cailloux, Grignon, and Joe (brook trout and white sucker lakes) were more numerous (creel census), had higher weight and higher percent recovery by sport-angling than native trout from lakes Deux Étapes and Hollis (brook trout lakes) (Table 2).

TABLE 1. General characteristics of the study lakes containing brook trout, and brook and white sucker populations.

Community	Lake	Area (ha)	Other fish species[a] PE	CCo	SM	Index of competition[b]
Brook trout	Deux Étapes	13.0				0
	Hollis	15.5				0
	Brochard	13.0	X			1
Brook trout	Cailloux	13.5	X	X		2
White sucker	Grignon	25.9	X	X	X	2
	Joe	23.3	X	X		2

[a]PE, northern redbelly dace (*Phoxinus eos*); CCo, white sucker (*Catostomus commersoni*); SM, pearl dace (*Semotilus margarita*).

[b]Competitors were assumed to be native brook trout (when abundance was higher than 0.3 fish captured/angler-day) and white sucker whose effects were additive. See text for derivation of index.

Planting

Each lake was stocked with equal numbers of each strain at a total rate of 150 fish/ha. This planting density seems acceptable for this type of lake (Fraser 1981). Plantings were carried out on October 7 and 8, 1985. The fish were transported by truck from the hatchery to Mastigouche Reserve in insulated oxygen-equipped tanks. Between 100–115 fish of each stock assigned to a specific lake were measured (± 1 mm TL) prior to planting, to ensure that subsamples (lakes) were representative of the total sample (hatchery). The fish were then transported in buckets and planted from a motor boat. The individuals were distributed all around the lake in shallow water, in the areas where cover was available. A 30 min portage was necessary to reach Lake Brochard. Consequently, fish were put in plastic bags (around 200 fish/bag) and filled the remaining volume with pure oxygen and a minimal amount of water. The plastic bags were then placed in back-packs and transported on foot.

Recovery

The six study lakes were open to sport angling from June 21 to July 12, 1986 and from June 20 to July 10, 1987. Fish captured by fishermen were identified to the strain by their finclip, weighed (± 0.01 g), measured (± 1 mm TL), and then the sex was determined. Since the fishing effort was not the same for all the lakes, results were expressed in percent recovery per angler-day to allow comparison among the different lakes and between the 2 yr of sampling. For each lake, the number of angler-days was determined by a complete creel check during the open season. We used a G-statistic to test the hypothesis that the three strains were recovered at the same frequency in each lake (Sokal and Rohlf 1981). When the hypothesis of homogeneity in recovering was rejected, we compared all possible pairs of strains using a simultaneous test procedure (STP; Sokal and Rohlf 1981), to determine which pairs of strains were homogeneous and which strains were significantly different

TABLE 2. Characteristics of the native brook trout found in the study lakes. Data are mean ± 1 SD in parentheses.

	Brook trout lakes					
	1986			1987		
	Deux Étapes Lake	Hollis Lake	Brochard Lake	Deux Étapes Lake	Hollis Lake	Brochard Lake
Number captured	11	10	42	2	5	27
Number captured/A.-D.[a]	0.3	0.2	1.3	0.1	0.1	0.9
Weight (g)	134.6 (37.1)	181.7 (50.6)	189.5 (134.5)	432.8 (230.9)	466.5 (51.8)	432.4 (158.1)
Length (mm)	238.8 (21.4)	264.6 (30.1)	329.4 (65.0)	330.0 (62.2)	355.2 (13.1)	340.1 (44.4)
Percent recovery[b]	2.7	2.4	24.6	1.5	2.1	12.4

	Brook trout and white sucker lakes					
	1986			1987		
	Cailloux Lake	Grignon Lake	Joe Lake	Cailloux Lake	Grignon Lake	Joe Lake
Number captured	70	182	163	37	60	66
Number captured/A.-D.[a]	1.7	3.9	4.1	0.9	1.6	1.9
Weight (g)	148.1 (79.6)	263.7 (109.9)	174.8 (77.6)	170.4 (111.6)	177.4 (70.0)	198.6 (154.5)
Length (mm)	244.4 (46.9)	313.6 (59.5)	258.0 (47.9)	255.6 (37.8)	255.4 (42.3)	262.0 (61.6)
Percent recovery[b]	31.8	55.7	58.2	29.1	20.0	52.8

[a]A.-D., Angler-Day.

[b]$\text{Percent recovery} = \frac{\text{Number of native fish captured by sport fishing}}{\text{Total number of fish captured by sport fishing}} \times 100.$

from the others to cause the heterogeneity. Computations for 1987 took into account the captures occurring in 1986 by sport fishing (reported in this study) and by experimental fishing (reported in Lachance and Magnan 1990).

Growth

Growth was analyzed by comparing the mean weight of each strain at stocking with their mean weight at capture. Since the wild strain was smaller at planting than the other two strains, and since the growth of fish is not isometric, we did not compare weights of the three strains at capture statistically.

The following three measures were used to interpret the results of growth for each strain: (1) mean weight at capture, (2) percent increment in weight, and (3) yield, in terms of kg of fish recovered by kg of fish planted. As for recoveries by angling, results were expressed in terms of yield per angler-day, to enable comparisons among the different lakes and between the 2 yr of sampling.

Impact of Competitors

All the parameters studied (i.e. recovery, growth, and yield) in brook trout lakes were compared to those of brook trout and white sucker lakes. This allowed us to verify if the white sucker had a significant impact on the brook trout with regard to one or more of these parameters, and to determine on which strain the impact was more important. Furthermore, as the density of native brook trout varied between the different study lakes, it was possible to investigate its impact on planted fish. To quantify the impact of intra- and interspecific competition on each strain, Pearson product-moment correlation coefficients were calculated between characteristics of fish communities (i.e. relative abundance of native brook trout, occurrence of white sucker, number of potential competitors; Tables 1 and 2) and response variables (i.e. recovery, percent of increment in weight, and yield). In these analyses, the presence or absence of white sucker were coded respectively 1 and 0, while the number of potential competitors was coded 0 (white sucker absent and relative abundance of native brook trout lower than 0.3 fish captured/angler-day), 1 (white sucker absent and relative abundance of native brook trout higher than 0.3 fish captured/angler-day), or 2 (white sucker present and relative abundance of native brook trout higher than 0.3 fish captured/angler-day; Tables 1 and 2).

Results

Recovery

In the first year after planting, 1986, recoveries were higher for domestic and hybrid strains in lakes containing no competitors (although the tendency was not significant in Lake Deux Étapes; Table 3). When white sucker and (or) native brook trout were present, the recoveries of the wild strain were significantly higher in lakes Brochard and Grignon, those of the hybrid strain were significantly higher in Lake Joe, and no significant differences were observed among recoveries of the three strains in Lake Cailloux (Table 3).

In the second year after planting, the recoveries were higher for the wild strain in five of the six study lakes (although the tendency was not significant in lakes Deux Étapes and Cailloux; Table 3). Also, it is noteworthy that the rates of recovery were always ordered in the same way in these five lakes; wild > hybrid > domestic (Table 3). In the sixth lake (Lake Joe), the recoveries of the hybrid strain were significantly higher than those of wild and domestic strains (Table 3).

Growth

In the first year following planting in brook trout lakes, the domestic strain had the highest mean weight at capture in lakes Deux Étapes and Hollis, and the hybrid strain in Lake Brochard (Table 4). In 1987, the hybrid strain had the highest mean weight at capture in all three lakes (Table 4). In the brook trout and white sucker lakes, the domestic strain had the highest mean weight at capture in all three lakes for both years (Table 4).

In 1986 in the brook trout lakes, the highest percent increment in weight was for the domestic strain in lakes Deux Étapes and Hollis, and for the wild strain in Lake Brochard (Table 4). In the second year following planting, the wild strain showed higher increment in weight in lakes Brochard and Hollis, and the hybrid strain in Lake Deux Étapes (Table 4). In the brook trout and white sucker lakes in the 2 yr following planting, the highest percent increment in weight was for the domestic strain in lakes Cailloux and Joe, and for the wild strain in Lake Grignon (Table 4). The percent increment in length, not presented in this paper, followed the same pattern as that of weight increment (Lachance 1988).

Yield

The highest yields in terms of kg of fish recovered per kg of fish planted were noted for the domestic strain in lakes Deux Étapes and Hollis, and for the wild strain in Lake Brochard, in the first year after planting (Table 4). In 1987, the highest yields were from the wild strain in lakes Brochard and Hollis, and from the hybrid strain in Lake Deux Étapes.

In the brook trout and white sucker lakes, the hybrid strain had the highest yield in the three lakes in 1986 (Table 4). In the second year following planting, the highest yields were from the wild strain in Lake Grignon, and from the hybrid strain in Lake Joe, while in Lake Cailloux the yields of the three strains were almost identical (Table 4).

Impact of Competitors

We observed that recovery, percent increment, and yield of planted fish were inversely correlated with (1) relative abundance of native brook trout, (2) the occurrence of white sucker and, (3) the number of potential competitors (Table 5). Brook trout performed better in the absence of competitors: over the 2 yr of the experiment, the overall mean percent recovery of planted brook trout was $4.80 \pm 1.17\%$ per angler-day in brook trout lakes compared to $1.14 \pm 0.77\%$ in lakes containing white sucker and native brook trout, the mean percent increment in weight was $3959 \pm 282\%$ compared to $1355 \pm 309\%$, and the mean yield, in terms of kg of fish recovered per kg of fish planted, was $83.9 \pm 6.3 \times 10^{-3}$ kg per angler-day compared to $11.0 \pm 8.7 \times 10^{-3}$ kg per angler-day, respectively. Lake Brochard (containing only native brook trout as competitor)

TABLE 3. Percent recovery by angling for the three strains of brook trout studied, during 2 yr after planting. DOM, domestic strain; HYB, hybrid strain; WILD, wild strain. For each lake, % recoveries with different letters are significantly different ($P < 0.05$) as determined with a G-test followed by an a posteriori simultaneous test procedure (STP; Sokal and Rohlf 1981).

	Brook trout lakes								
	Deux Étapes Lake			Hollis Lake			Brochard Lake		
	DOM	HYB	WILD	DOM	HYB	WILD	DOM	HYB	WILD
Number planted	650	650	650	775	775	775	650	650	650
Number recovered 1986	137	136	122	175	153	84	43	24	62
(Number angler-day)		(41)			(43)			(33)	
% Recovery/A.-D.$(10^{-1})^a$	5.14a	5.10a	4.58a	5.25a	4.59a	2.52b	2.00ab	1.12a	2.89b
Number recovered 1987	35	43	55	54	72	107	43	51	97
(Number angler-day)		(17)			(34)			(31)	
% Recovery/A.-D.$(10^{-1})^b$	4.96a	6.78a	8.09a	2.65a	3.40ab	4.55b	2.28a	2.62a	5.32b
	Brook trout and white sucker lakes								
	Cailloux Lake			Grignon Lake			Joe Lake		
	DOM	HYB	WILD	DOM	HYB	WILD	DOM	HYB	WILD
Number planted	650	650	650	1295	1295	1295	1165	1165	1165
Number recovered 1986	49	57	44	25	39	81	31	60	26
(Number angler-day)		(41)			(47)			(40)	
% Recovery/A.-D.$(10^{-1})^a$	1.77a	2.06a	1.59a	0.41a	0.64a	1.33b	0.66a	1.29b	0.56a
Number recovered 1987	23	27	40	20	64	156	12	34	13
(Number angler-day)		(41)			(38)			(34)	
% Recovery/A.-D.$(10^{-1})^b$	0.91a	1.09a	1.57a	0.41a	1.34b	3.38c	0.31a	0.90b	0.34a

$$^a\%\ \text{Recovery/A.-D. 1986} = \frac{\text{Number recovered by angling (1986)}}{\text{Number planted (1985)}} \times 100.$$

$$^b\%\ \text{Recovery/A.-D. 1987} = \frac{\text{Number recovered by angling (1987)}}{\text{Number planted (1985)} - \text{Number recovered by angling (1986)}} \times 100.$$

TABLE 4. Mean weight (g ± 1 SD in parentheses) at planting and at recovery by angling, mean percent increment of weight and yield for the three strains of brook trout studied, during 2 yr after planting. DOM, domestic strain; HYB, hybrid strain; WILD, wild strain.

	Brook trout lakes								
	Deux Étapes Lake			Hollis Lake			Brochard Lake		
	DOM	HYB	WILD	DOM	HYB	WILD	DOM	HYB	WILD
Weight (g) 10/10 1985[a]	12.8 (4.4)	11.9 (4.5)	10.3 (3.9)	13.3 (4.1)	13.9 (4.3)	11.3 (3.6)	14.8 (3.6)	14.7 (4.5)	10.5 (3.4)
Weight (g) 21/06 to 12/07 1986	168.9 (40.7)	151.7 (41.7)	107.7 (27.6)	216.6 (52.6)	199.8 (51.6)	124.7 (35.0)	96.1 (27.2)	98.6 (26.6)	74.0 (19.2)
Number of fish	126	128	109	162	139	77	43	24	62
% Increment 1986[b]	1219.5	1174.8	945.6	1528.6	1337.4	1003.5	549.3	570.7	604.8
Yield/A.-D.($\times 10^{-3}$) 1986[c]	62.4	61.2	42.8	79.2	59.9	25.5	13.0	7.5	20.4
Number angler-day		41			43			33	
Weight (g) 20/06 to 10/07 1987	513.6 (82.9)	533.1 (81.9)	408.9 (85.8)	494.1 (57.3)	519.9 (78.6)	433.6 (78.2)	274.6 (42.1)	283.2 (50.8)	239.2 (39.5)
Number of fish	35	43	55	54	72	107	43	51	97
% Increment 1987[b]	3912.5	4379.8	3869.9	3615.0	3640.3	3737.1	1755.4	1826.5	2178.1
Yield/A.-D.($\times 10^{-3}$) 1986 plus 1987[d]	81.3	94.4	88.1	77.8	78.6	83.0	25.9	26.5	63.6
Number angler-day 1986 plus 1987		58			77			64	

	Brook trout and white sucker lakes								
	Cailloux Lake			Grignon Lake			Joe Lake		
	DOM	HYB	WILD	DOM	HYB	WILD	DOM	HYB	WILD
Weight (g) 10/10 1985[a]	13.3 (4.2)	13.3 (4.4)	10.7 (3.9)	14.4 (4.5)	14.9 (4.1)	10.1 (3.5)	12.6 (4.0)	13.3 (3.7)	10.6 (3.9)
Weight (g) 21/06 to 12/07 1986	71.6 (15.7)	66.6 (16.8)	49.3 (15.7)	82.5 (16.7)	80.7 (22.9)	61.7 (21.0)	68.0 (21.4)	59.1 (11.1)	51.1 (13.6)
Number of fish	49	56	44	25	154	81	31	57	25
% Increment 1986[b]	483.3	400.7	360.7	472.9	441.6	510.9	439.7	344.4	382.1
Yield/A.-D.($\times 10^{-3}$) 1986[c]	9.5	10.1	7.3	2.6	13.7	8.1	3.6	5.4	2.6
Number angler-day		41			47			40	
Weight (g) 20/06 to 10/07 1987	192.4 (79.3)	167.8 (30.9)	113.6 (21.4)	244.2 (54.3)	232.7 (43.3)	188.2 (39.2)	159.1 (91.6)	145.7 (35.9)	100.2 (30.6)
Number of fish	23	27	40	20	64	156	12	34	13
% Increment 1987[b]	1346.6	1161.6	961.7	1595.8	1461.7	1763.4	1162.7	995.5	845.3
Yield/A.-D.($\times 10^{-3}$) 1986 plus 1987[d]	10.8	11.2	11.3	4.4	16.7	30.9	3.7	7.3	2.8
Number angler-day 1986 plus 1987		82			85			74	

[a]Mean weight (g) at planting estimated from a length-weight relationship (see text).

[b]$\frac{\text{Mean weight at capture} - \text{mean weight at planting}}{\text{Mean weight at planting}} \times 100.$

[c]$\frac{\text{Biomass captured by sport fishing in 1986}}{\text{Biomass planted in 1985}}$ /A.-D.

[d]$\frac{\text{Biomass captured by sport fishing in 1987 and 1986}}{\text{Biomass planted in 1985}}$ /A.-D.

TABLE 5. Pearson product-moment correlation coefficients between characteristics of fish communities and response variables. NS, non-significant correlation at $P \geq 0.05$ (α_1).

Response variable	Strain	Relative abundance of native brook trout	Occurrence of white sucker	Number of potential competitors
Percent recovery 1986	DOM	−0.91	−0.81	−0.96
	HYB	−0.81	−0.65 NS	−0.65 NS
	WILD	−0.82	−0.84	−0.84
1987	DOM	−0.86	−0.85	−0.90
	HYB	−0.79	−0.78	−0.87
	WILD	−0.76	−0.84	−0.82
Percent increment (g) 1986	DOM	−0.78	−0.74	−0.92
	HYB	−0.81	−0.80	−0.96
	WILD	−0.78	−0.85	−0.97
1987	DOM	−0.88	−0.78	−0.95
	HYB	−0.88	−0.80	−0.96
	WILD	−0.86	−0.86	−0.97
Yield 1986	DOM	−0.81	−0.76	−0.94
	HYB	−0.74	−0.68 NS	−0.90
	WILD	−0.82	−0.86	−0.92
1987	DOM	−0.92	−0.84	−0.98
	HYB	−0.88	−0.80	−0.96
	WILD	−0.86	−0.94	−0.96

presented an intermediate situation: the mean percent recovery of planted brook trout was 2.71 ± 1.42%, the mean percent of increment in weight was 2032 ± 220%, and the mean yield was $38.7 \pm 21.6 \times 10^{-3}$ kg per angler-day.

The impact of these competitors did not appear to be more important for one strain than another over the 2 yr of the experiment, in terms of percent recovery (Table 3). However, the fact that the domestic strain was recovered in lower proportion than the wild and hybrid strains in lakes Brochard, Cailloux, Grignon, and Joe in 1986, could be explained by the higher densities of native trout there; domestic strain did best in their first year in lakes with fewest native trout (Deux Étapes and Hollis; Table 3).

Discussion

Performance of the Strains Studied

The results of this 2 yr study indicated that either in the absence or presence of competitors, the wild strain performed better than the domestic strain after stocking, and that the hybrid strain showed an intermediate performance. During the first year after stocking, recovery, mean percent increment in weight, and yield of domestic and/or hybrid strains were highest in four of the six study lakes. In contrast, during the second year after stocking, the wild and hybrid strains showed the highest rates of recovery and yield in the six study lakes, despite individuals of domestic strain having higher percent of increment in weight in two lakes. The smaller size of wild strain at planting might have affected their recovery and yield in the first year but not in the second year of the experiment. However, although size at planting did not apparently affect survival, it had an impact on growth of the stocked fish; the advantage in weight of domestic and/or hybrid strains at planting was maintained over the 2 yr. These results agree with those of Greene (1952), Vincent (1960), Flick and Webster (1964, 1976), Fraser (1981), Keller and Plosila (1981), and Webster and Flick (1981). Domestic strains generally survive for a shorter time, or are captured more rapidly than the wild and hybrid strains. However, their greater size at stocking provides good yields in the first year after planting in lakes containing few competitors. In contrast, the wild and hybrid strains generally survive longer and the hybrid strain have a percent of increment in weight often higher than that of domestic strains. These two factors allow them to reach greater size, which gives better yield on the longer term. It is noticeable that the performances of the three strains were comparable in all the above-cited papers although the studies were done in different habitats and with trout originating from different stocks.

Different hypotheses are suggested to explain this lower survival in domestic strains. The first is a poor adaptability of individuals to the available food resources in the field, which causes mortality through starvation (Miller 1952; Ersbak and Haase 1983). Starvation cannot apparently explain the lower survival of individuals in our study because brook trout of domestic strain had a similar diet (in quality and quantity) to that of hybrid and wild strains (Lachance and Magnan 1990). Furthermore, we observed that growth of domestic individuals in the first year after planting was among the highest, suggesting that energetic supplies were adequate. However, if there was large individual variability in the ability to find food in the field, it is possible that starvation had occurred soon after stocking. In this context, the lower recovery of domestic strain in the second year after stocking might have been explained by a higher susceptibility to capture in the first year (because of their size or behaviour). A second hypothesis is a higher natural mortality of the domestic strain in the second winter. These individuals were recovered at a lower rate than those of the hybrid strain in the second year, although they were recovered at similar rates in the first year. Earlier maturation of domestic, and to some extent of hybrid strains (Lachance and Magnan 1990) may have increased their overall mortality, and reduced survival to the second year.

A third hypothesis to explain the lower survival in domestic strains is a higher susceptibility to predation (Vincent 1960). In a laboratory experiment using fish reared in similar conditions, Vincent (1960) observed that individuals of a domestic strain preferred the higher part of the water column and did not flee when a hand was moved over the tank. In contrast, individuals of a wild strain preferred the lower part of the water column and fled rapidly at any movement above the tank. In the field, this behaviour of domestic strains may render them more susceptible to predation by native fish, birds, and mammals. A study on spatial distribution of fish in two of our six study lakes indicated that all three strains were mainly captured in the lower part of the water column, in the littoral and offshore zones (Lachance and Magnan 1990). However, as suggested by the first hypothesis, such a predation from native fish, birds, and mammals might have occurred only soon after stocking. Planted fish were presumably too large after the first winter in the field to be preyed upon, an assumption supported by stomach content analysis (Lachance and Magnan 1990).

A fourth hypothesis is that the better resistance to the stress of planting and to the life under field conditions, inherent to wild and hybrid strains, is responsible for their higher survival (Vincent 1960). We have no data relevant to this hypothesis.

Impact of Competitors

The results of this study indicate that white sucker and native brook trout had an impact on the performance of the three strains

studied. The recovery, percent increment in weight, and yield were inversely correlated with the relative abundance of native brook trout and with the occurrence of white sucker, indicating that both intra- and interspecific competitions were significant. Furthermore, the response variables were also inversely correlated with the number of potential competitors, suggesting that the impact of native brook trout and white sucker were additive. Alternatively, differences in response variables might be due to differences in size of the study lakes; percent recoveries were lower in the two larger lakes (Grignon and Joe) than in the other four lakes, in the first year after planting. However, this tendency was not maintained in the second year, and did not occur for the other parameters (percent increment and yield). Differing densities in large and small lakes were not a factor because fish were stocked at equal rates in each lake (i.e. 150/ha).

The mechanisms responsible for this lower performance of planted brook trout in the presence of white sucker and/or native brook trout might be competition for available food resources. For example, we observed that the feeding habits of brook trout planted in brook trout and white sucker lakes differed from those planted in brook trout lakes, suggesting a niche shift of the trout when living with white sucker (Lachance and Magnan 1990). Niche shifts are strong evidence that competition occurs in natural communities, and are identified as a mechanism which allows for a decrease in the intensity of competition (Diamond 1978; Schoener 1982; Werner 1986). Earlier work in the same study system showed that the presence of white sucker caused a niche shift in natural brook trout populations with respect to spatial distribution and feeding habits (Magnan 1988; S. Tremblay and P. Magnan, unpubl. data) and significantly reduced their mean annual yield, in terms of kilograms of fish captured by angling per hectare (Magnan 1988). We hypothetize that starvation due to intra- and/or interspecific competition for available food resouces involved high mortality of planted fish soon after stocking.

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Comparative Ecology and Behavior of Domestic, Hybrid, and Wild Strains of Brook Trout,[1] *Salvelinus fontinalis*, after Stocking

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Two years after planting in six small oligotrophic lakes, domestic, hybrid and wild strains of brook trout, *Salvelinus fontinalis*, used space and food resources in the same way as native trout. Although trout living with or without white sucker, *Catostomus commersoni*, were similarly spatially distributed, they had different diets, suggesting a feeding niche shift of trout in the presence of sucker; we concluded that this shift is under phenotypic control because each planted strain came from similar genetic backgrounds. Sexual maturity was related to the size of individuals, regardless of the strain, and males matured before females. Almost all males and females were sexually mature in the first fall after stocking except wild females (3.0 and 75% matured during the first and second fall, respectively). Gonadosomatic indices (GSI) of domestic and hybrid females were similar during the first fall, but the GSI of domestic females was significantly higher than that of hybrid and wild strains in the second fall. Mean egg diameters were similar among the three strains during the two falls, but fecundity of domestic females, after correction for size differences, was significantly higher than that of hybrid females which, in turn, was significantly higher than that of wild ones.

Deux ans après leur ensemencement dans six petits lacs oligotrophes, des lignées domestique, hybride et sauvage d'omble de fontaine, *Salvelinus fontinalis*, utilisaient l'espace et les ressources alimentaires de la même façon qu'une lignée indigène d'omble. Quoique les ombles vivant avec ou sans meunier noir, *Catostomus commersoni*, avaient une distribution spatiale similaire, ils avaient une alimentation différente, suggérant un déplacement de niche alimentaire de l'omble en présence du meunier; nous avons conclu que ce déplacement est sous contrôle phénotypique étant donné que chaque lignée provenait d'une même souche génétique. La maturité sexuelle était reliée à la taille des individus pour toutes les lignées et les mâles étaient matures avant les femelles. Presque tous les mâles et les femelles étaient matures sexuellement durant le premier automne après l'ensemencement à l'exception des femelles sauvages (3.0 et 75% étaient matures durant les premier et second automnes, respectivement). L'indice gonadosomatique (IGS) des femelles domestiques et hybrides étaient similaires durant le premier automne, mais l'IGS des femelles domestiques était significativement plus grand que celui des femelles hybrides et sauvages durant le second automne. Le diamètre moyen des oeufs était similaire chez les trois lignées durant les deux automnes, mais la fécondité des femelles domestiques, après correction pour les différences de taille, était significativement plus élevée que celle des femelles hybrides qui elle, était significativement plus élevée que celle des femelles sauvages.

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Numerous studies have investigated the performance of different strains of brook trout, *Salvelinus fontinalis*, after stocking (Flick and Webster 1964, 1976; Fraser 1972, 1978, 1980, 1981; Keller and Plosila 1981; Webster and Flick 1981; Lachance and Magnan 1990). Paradoxically, very few have investigated the ecology and behavior of the fish after planting. One exception is Fraser (1981) who compared the feeding habits of wild, hybrid and domestic strains of brook trout in some Ontario lakes. In a recent analysis, Wydoski (1986) stressed the importance of understanding the behavior and certain ecological traits of the fish after release in the field to improve stocking as well as to create, enhance and restore cold-water fisheries.

The first objective of the present study was to compare the feeding habits, spatial distribution and reproductive traits of domestic, hybrid and wild strains of brook trout during 2 yr after stocking in six small oligotrophic lakes of the Laurentian Shield. Native brook trout found in these lakes were also studied for comparison. A second objective was to investigate the impact of white sucker, *Catostomus commersoni*, present in three of the lakes, on the feeding habits and spatial distribution of planted brook trout. S. Tremblay and P. Magnan, unpubl. data and Magnan (1988) observed that natural populations of brook trout shifted their spatial distribution from the littoral to the offshore zone, and their feeding habits from zoobenthos to zooplankton when living in sympatry with white sucker.

Materials and Methods

Summary of Materials and Experimental Design

The three strains of brook trout were reared in Government hatcheries to the fall fingerling stage at which time they were fin-clipped for subsequent identification of the strain in the

[1]The authors prefer the common name "charr" to designate species of the genus *Salvelinus*.

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field. The six study lakes were stocked with equal numbers of each strain at a total rate of 150 fish/ha, on October 7 and 8, 1985. Lakes were divided into two groups, those containing native brook trout with or without northern redbelly dace, *Phoxinus eos*, called "brook trout lakes" (Deux Étapes, Hollis, and Brochard), and those containing native brook trout, white sucker and northern redbelly dace, "brook trout and white sucker lakes" (Cailloux, Grignon, and Joe). Almost all lakes contained northern redbelly dace but their presence did not significantly affect the mean yield of brook trout in the exploited lakes of the Mastigouche Reserve (P. Magnan, unpubl. data). Native brook trout reported in the present paper did not come from self-sustaining populations to sport fishing exploitation (i.e. in lakes where brook trout are unable to sustain fishing exploitation without any fish stocking; see Magnan 1988). Further details on the origin of the brook trout strains, characteristics of the study lakes and planting of trout may be found in Lachance and Magnan (1990), which describes a concurrently performed study.

Feeding Habits

Stomach contents were removed from brook trout (both native and planted) captured by sport fishermen and preserved in a 10% formaldehyde solution. The part of the digestive tract situated between the oesophagus and the pyloric valve was analyzed (Magnan and FitzGerald 1982) from a total of 20 individuals per strain (when the number of captures allowed), on all the lakes between June 21 and July 12, 1986, and on lakes Hollis and Cailloux between June 20 and July 10, 1987. Organisms were generally identified to order and weighed (± 0.1 mg) before computing the mean percent weight of each prey found in the stomach (Hyslop 1980; Walsh and FitzGerald 1984). For interpretation, the different taxonomic groups were pooled according to the following functional groups: zooplankton (Cladocera, Chaoboridae larvae, Diptera pupae), benthic organisms (captured on the bottom), mature insects (captured at the surface or in the water column during emergence), fish and other preys (including molluscs, Hirudinae, and debris of insects). Finally, the fullness index (FI) of each stomach was estimated by dividing the wet weight of the stomach contents (g) by the length (mm) of the corresponding fish (Hyslop 1980; Walsh and FitzGerald 1984). This index indicates the satiation level of the fish. The mean fullness index of stomachs of the three strains were compared for each lake using an analysis of variance (ANOVA) followed by an a posteriori Student–Newman–Keuls multiple-range comparison test (SNK). The homogeneity of the variances was tested prior to analyses using a F-max test (Sokal and Rholf 1981) and the data for which the variances were not homogeneous were log $(x + 1)$ transformed.

Spatial Distribution

Gill netting was carried out in the littoral zone (0–2 m depth) and in the offshore zone (0–4 m depth) of Lake Deux Étapes and Lake Cailloux in 1986 and of Lake Hollis and Lake Cailloux in 1987. Sampling took place in the first week of August 1986 and 1987, a period when food resources were presumably less abundant in such habitats (Werner 1986), and therefore, when movements should have been greater.

Fish were captured with an experimental multifilament gill net (1.8 m deep × 38.1 m long) with stretched mesh of 2.5, 3.8, 5.1, 6.4, and 7.6 cm. In the littoral zone, the nets were arbitrarily placed in 1.5–2.0 m depth, parallel to the shore, whereas in the offshore zone, two nets were superimposed in the water column, giving an effective fishing gear 3.6 m deep × 38.1 m long. The position of the fish in the water column (± 5 cm) and the length (± 1 mm TL) of all individuals were noted. Gill nettings were carried out until 30 fish of each strain were caught or until five 12-h periods passed by for each lake. In 1987, the number of captures at Lake Cailloux was too low to allow for a valid analysis. The nets were examined twice a day (at sunrise and sunset) to provide comparable fishing periods.

Spatial distribution of the fish was analyzed with exact probabilities of the binomial (Sokal and Rohlf 1981). We tested more specifically the two following null hypotheses: (1) there are no significant differences in the spatial distribution of the fish between the littoral and offshore zones and (2) there are no significant differences in the vertical distribution of the fish within the upper and lower part of the water column. We considered the lower part of the water column to be between 1.5 and 2.0 m depth in the littoral zone, and between 3.0 and 4.0 m depth in the offshore zone. The upper part of the water column was from 1.5 m depth to the surface in the littoral zone, and from 3.0 m depth to the surface in the offshore zone. For statistical analysis, the expected frequencies were determined on the basis of fishing effort carried out in each zone and part of the water column (i.e. littoral vs. offshore zones and upper part vs. lower part of the water column; e.g. Hume and Northcote 1985). In this way, if we had two gill nets set in the littoral zone and one in the offshore zone, the theoretical (expected) ratio was 2:1 for the testing of hypothesis 1. The expected frequencies were always 3:1 for hypothesis 2 since fishing effort was three times higher in the upper part than in the lower part of the water column.

Reproductive Potential

To evaluate the reproductive potential of the different strains, gill netting was carried out on Lake Deux Étapes in September 1986 and 1987, just before the brook trout spawning season at this latitude (Scott and Crossman 1973). This lake, a brook trout lake, was assumed to provide favorable conditions for reproduction since the Ministère du Loisir, de la Chasse et de la Pêche du Québec built an artificial spawning ground there in 1985. In addition, almost all brook trout reached the potential size of sexual maturity in this lake (around 170 mm for this species; see Magnan and FitzGerald 1983) during the first year after planting. No investigation was done in the brook trout and white sucker lakes since the planted trout were too small to be mature in 1986 and the number of recoveries was too low in 1987. Nets were set until about 50 fish of each strain were captured. Each individual was identified to strain, measured (± 1 mm TL), weighed (± 0.01 g), and the gonadosomatic index (GSI) was calculated using the formula : GSI = (gonad weight/body weight) × 100 (McCormick and Naiman 1984).

In addition, the mean egg diameter of each mature female was estimated from the measure of three subsamples of 10 eggs (± 0.01 mm). Finally, the fecundity of each female was evaluated by gravimetry (Snyder 1983). Here, the total number of eggs was estimated by proportionality from the mean weight of three subsamples of 25 eggs and the weight of the remaining eggs. The eggs were weighed (± 0.1 mg) following a 24 h free air drying period. This method was accurate at $\pm 1\%$ when compared to the total count carried out on 10% of the samples.

TABLE 1. Mean percent weight of prey found in stomach contents of brook trout in the first year after planting (1986). DOM, domestic strain; HYB, hybrid strain; WILD, wild strain; NAT, native brook trout.

	Brook trout lakes											
	Deux Étapes Lake				Hollis Lake				Brochard Lake			
	DOM	HYB	WILD	NAT	DOM	HYB	WILD	NAT	DOM	HYB	WILD	NAT
Number of stomachs	21	20	19	8	19	20	20	9	20	20	20	20
Mean length (± 1 SD) (mm)	251.4 (17.8)	256.8 (19.0)	224.4 (23.7)	233.2 (16.9)	266.2 (18.1)	276.1 (15.5)	235.9 (19.1)	259.6 (27.1)	210.4 (17.1)	214.6 (14.4)	193.9 (15.6)	351.9 (40.0)
Zooplankton[a]												
Cladocera	26.1	19.4	0.1	20.4	31.0	18.9	7.4	29.3	0.0	0.0	0.0	0.0
Chaoboridae(L)	4.0	5.6	8.5	1.0	1.0	2.2	0.2	0.2	0.7	4.7	0.2	7.1
Diptera (P)	4.7	2.0	6.2	0.8	1.0	0.5	15.0	0.5	22.5	7.1	13.9	12.5
Total	34.8	27.1	14.7	22.3	32.9	21.6	22.6	30.0	23.2	11.8	14.1	19.6
Benthic organisms												
Ephemeroptera(L)	18.6	13.7	25.6	23.7	15.0	8.2	14.4	23.5	0.0	0.0	2.3	0.0
Odonata (L)	3.6	19.9	16.9	19.9	20.4	24.9	10.2	10.0	4.0	2.1	3.4	9.9
Trichoptera (L)	10.1	14.9	6.0	8.6	0.5	5.8	5.6	0.4	0.3	0.5	0.0	0.2
Others[b]	7.8	0.6	0.0	0.1	0.1	0.4	4.4	2.2	0.0	0.9	0.0	5.2
Total	40.1	49.1	48.5	52.3	36.0	39.3	34.6	36.1	4.3	3.5	5.9	15.3
Adult insects												
Coleoptera	7.2	12.6	19.0	18.8	6.3	3.5	12.1	11.3	7.3	14.5	12.4	2.4
Odonata	7.2	5.0	1.0	0.0	0.6	5.7	4.5	16.6	20.1	20.5	24.2	37.0
Trichoptera	4.0	2.9	7.9	4.2	13.2	23.4	18.1	0.6	38.3	43.2	35.0	11.0
Others	5.5	2.0	5.2	2.4	0.1	1.4	1.3	4.3	4.1	4.9	2.7	0.7
Total	23.8	22.5	33.1	25.4	20.2	34.0	36.0	32.8	69.8	83.1	74.3	51.1
Fish	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	9.5
Miscellaneous	1.3	1.4	3.7	0.0	5.8	5.1	6.5	1.1	2.7	1.6	5.7	4.0
F.I. $\times 10^{-3}$(g)[c]	2.5a (2.3)	1.3b (0.9)	2.6a (2.2)	2.5a (1.7)	2.4a (2.4)	2.4a (1.7)	1.2b (1.4)	3.1a (3.7)	5.5a (3.4)	4.7a (3.9)	3.5b (2.6)	6.4c (5.5)

	Brook trout and white sucker lakes											
	Cailloux Lake				Grignon Lake				Joe Lake			
	DOM	HYB	WILD	NAT	DOM	HYB	WILD	NAT	DOM	HYB	WILD	NAT
Number of stomachs	17	20	20	20	18	22	20	20	20	20	20	20
Mean length (± 1 SD) (mm)	199.8 (9.6)	199.4 (16.2)	181.1 (16.3)	248.3 (40.1)	205.8 (14.2)	207.6 (19.0)	185.0 (20.6)	337.0 (48.2)	193.3 (18.4)	179.4 (13.0)	174.9 (15.2)	277.0 (29.3)
Zooplankton[a]												
Cladocera	24.6	32.2	48.2	0.7	34.4	12.2	31.7	2.1	0.0	4.7	1.7	4.3
Chaoboridae(L)	2.1	5.0	0.0	2.3	0.0	0.0	0.7	23.9	0.2	0.1	0.0	14.7
Diptera (P)	2.6	1.1	0.5	0.4	4.4	1.0	4.2	17.9	0.4	0.4	2.0	7.3
Total	29.3	38.3	48.7	3.3	38.8	13.2	35.6	43.9	0.6	5.2	3.7	26.3
Benthic organisms												
Ephemeroptera (L)	2.5	19.5	33.0	34.8	9.2	9.2	12.3	6.1	38.9	60.7	53.8	14.5
Odonata (L)	0.0	1.3	0.8	11.5	11.1	26.0	10.9	5.7	0.6	0.0	2.4	4.9
Trichoptera (L)	0.8	0.0	0.4	0.3	0.4	0.4	0.1	0.8	0.7	1.2	0.3	0.2
Others[b]	2.4	0.4	1.3	1.8	0.8	1.4	4.5	5.4	1.1	0.2	0.0	3.9
Total	5.7	21.2	35.5	48.4	21.4	37.0	27.7	18.0	41.3	62.1	56.5	23.5
Adult insects												
Coleoptera	22.8	23.7	5.6	1.1	22.6	23.7	20.5	21.1	41.4	19.1	26.4	25.0
Odonata	1.4	2.4	0.0	4.1	7.6	7.1	4.5	0.3	0.9	0.0	0.0	0.1
Trichoptera	0.0	0.5	0.1	0.3	0.9	3.9	0.6	3.9	1.5	0.3	1.5	0.0
Others	19.6	3.6	3.4	10.1	1.9	8.3	5.6	4.2	12.9	9.2	11.9	3.3
Total	43.8	30.2	9.2	15.7	33.0	43.0	31.3	29.4	56.8	28.6	39.8	28.4
Fish	13.6	0.0	0.0	29.0	0.0	0.0	0.0	4.7	0.0	2.3	0.0	16.1
Miscellaneous	7.6	10.4	6.7	3.6	6.8	6.8	4.5	4.0	1.3	1.8	0.0	5.8
F.I. $\times 10^{-3}$(g)[c]	1.6a (2.5)	0.8b (0.8)	1.6a (1.2)	2.7c (2.2)	5.1a (4.1)	2.2b (2.8)	1.2b (1.1)	1.9b (4.0)	2.0a (1.5)	1.3b (1.2)	2.7a (2.3)	2.4a (2.9)

[a]P, pupa; L, larvae.
[b]Others; Mollusca, Hirudinae, and insect debris.
[c]F.I.; Mean fullness index (g) ± 1 SD in parentheses. Means with different letters are significantly different as determined by an ANOVA followed by a Student–Newman–Keuls multiple-range comparison test ($P<0.05$).

TABLE 2. Mean percent weight of prey found in stomach contents of brook trout in the second year after planting (1987). DOM, domestic strain; HYB, hybrid strain; WILD, wild strain; NAT, native brook trout.

	Brook trout lake Lake Hollis				Brook trout and white sucker lake Lake Cailloux			
	DOM	HYB	WILD	NAT	DOM	HYB	WILD	NAT
Number of stomachs	19	19	19	0	18	20	20	20
Mean length (± 1 SD) (mm)	358.4 (15.3)	370.8 (22.5)	354.4 (24.6)	—	265.5 (31.0)	261.2 (17.2)	233.5 (13.1)	264.1 (47.3)
Zooplankton[a]								
Cladocera	19.2	8.0	9.7	—	0.0	12.2	20.2	5.0
Chaoboridae(L)	2.4	9.3	16.9	—	2.7	0.4	0.8	10.3
Diptera (P)	1.4	9.0	2.1	—	1.4	1.8	3.2	0.4
Total	23.0	26.2	28.7	—	4.1	14.4	24.1	15.7
Benthic organisms								
Ephemeroptera (L)	7.2	28.8	12.5	—	32.5	30.2	34.7	21.4
Odonata (L)	13.5	6.9	12.6	—	6.8	9.8	10.1	5.6
Trichoptera (L)	4.8	0.0	0.0	—	0.0	0.0	0.0	0.0
Others[b]	2.9	3.3	12.4	—	0.1	0.0	0.9	0.8
Total	28.4	39.0	37.5	—	39.4	40.0	45.7	27.8
Adult insects								
Coleoptera	2.1	1.5	1.1	—	4.6	10.7	2.5	0.1
Odonata	9.6	17.7	6.4	—	1.7	1.6	0.0	3.9
Trichoptera	8.4	0.2	2.4	—	0.0	0.2	0.0	0.2
Others	2.1	0.1	2.2	—	5.5	8.0	3.7	0.9
Total	22.1	19.5	12.0	—	11.8	20.5	6.2	5.0
Fish	0.0	0.0	0.0	—	32.4	13.0	12.5	48.4
Miscellaneous	26.4	23.8	21.8	—	12.3	12.1	11.5	3.2
F.I. $\times 10^{-3}$(g)[c]	1.7a (1.7)	2.9b (1.8)	1.2a (1.2)	—	3.8a (2.5)	7.6b (7.2)	5.4a (5.0)	7.7b (9.5)

[a]P, pupa; L, larvae.
[b]Others; Mollusca, Hirudinae, and insect debris.
[c]F.I.; Mean fullness index (g) ± 1 SD in parentheses. Means with different letters are significantly different as determined by an ANOVA followed by a Student–Newman–Keuls multiple-range comparison test ($P<0.05$).

Linear regressions between log (fecundity) and log (female weight) were significant for all the strains and both years of the study ($P < 0.05$). However, the slopes of the equations for all strains did not differ significantly within each year ($P \geq 0.05$). Therefore, to remove the effect of size of females on fecundity, we used an analysis of covariance (ANCOVA), with weight as covariate, to compare the adjusted mean fecundity among strains (Sokal and Rohlf 1981). This analysis tests the main null hypothesis that there are no significant differences among sampled means Y_i (fecundity) when these are adjusted for a common mean of X (weight) and a common regression line (Sokal and Rohlf 1981). Values of fecundity were adjusted for a grand mean total weight of 207.4 g in 1986, and of 605.9 g in 1987. Because there were more than two strains to compare in 1987, differences among adjusted mean fecundities were determined using the a posteriori approximate comparison intervals method of Gabriel (Sokal and Rohlf 1981).

The egg diameter and the GSI, which were not correlated with length or weight of females, were compared using a t-test in 1986, and with an ANOVA followed by an a posteriori SNK multiple-range comparison test in 1987, since in the latter case, there were more than two strains compared. The gonadosomatic index of the males were compared using an ANOVA followed by an a posteriori SNK test in 1986 and 1987 (Sokal and Rohlf 1981). The homogeneity of the variances were tested using a F-max test (Sokal and Rohlf 1981), and data for which the variances were not homogeneous were log (x) transformed.

Results

Feeding Habits

Both native and planted brook trout had a similar diet within each of the lakes containing few competitors (i.e. lakes Deux Étapes and Hollis where relative abundance of native trout was less than 0.3 fish captured/angler-day; see Lachance and Magnan 1990 for discussion on competition in these lakes). In 1986, their diet consisted mainly of benthic organisms (Ephemeroptera and Odonata larvae), mature insects (Coleoptera, Trichoptera, and Odonata) and zooplankton (Cladocera), with the exception of the wild strain individuals which fed less on Cladocera but more on Chaboridae larvae and/or Diptera pupae (Table 1). In 1987, the diet of planted brook trout found in Lake Hollis was comparable to that observed in 1986. The individuals fed chiefly on benthic organisms (Ephemeroptera and Odonata larvae), zooplankton, and mature insects (Odonata) (Table 2). The stomachs from the five native brook trout captured in 1987 were empty.

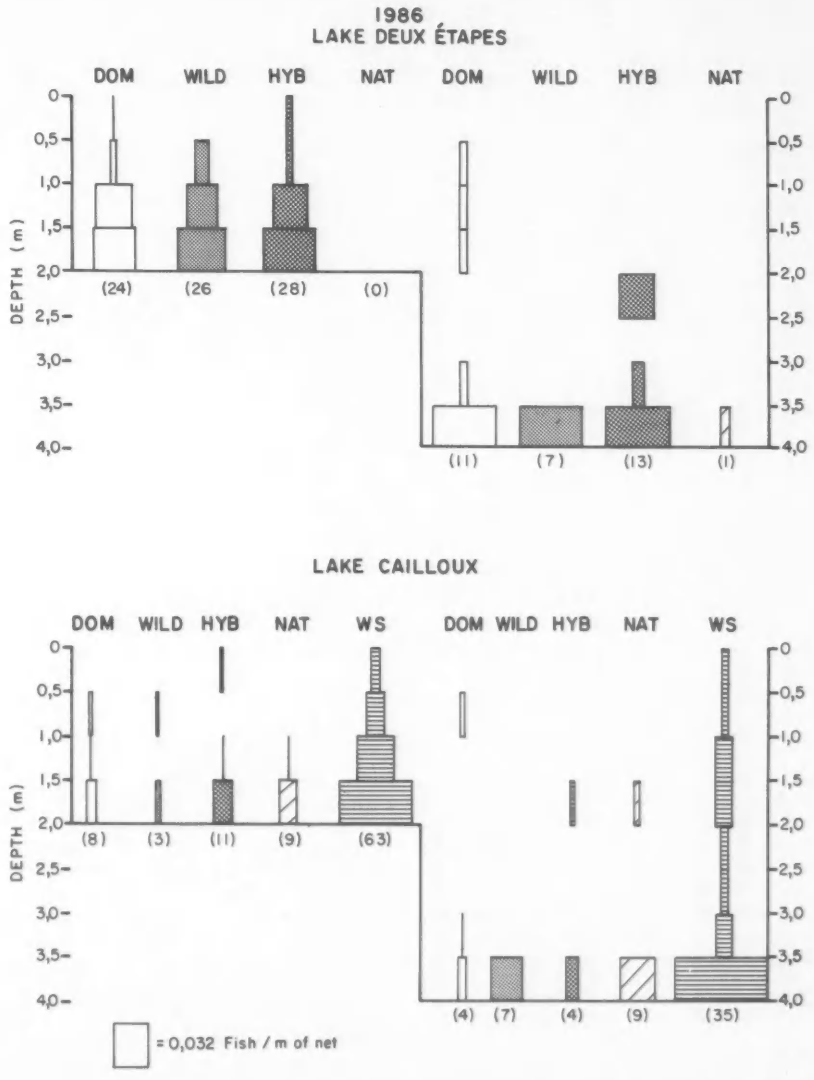

FIG. 1. Spatial distribution of brook trout and white sucker in the littoral (2-m contour) and offshore (4-m contour) zones in lakes Deux Étapes (brook trout lake) and Cailloux (brook trout and white sucker lake) during the first summer after stocking (1986). DOM, domestic strain; WILD, wild strain; HYB, hybrid strain; NAT, native brook trout; WS, white sucker. Number of captures are in parentheses.

In contrast, major differences were noted between the feeding habits of planted and native brook trout in the first year following planting in the lakes containing competitors (i.e. with white sucker and/or a relative abundance of native brook trout higher than 0.3 fish captured/angler-day; see Lachance and Magnan 1990). In Lake Brochard, where native brook trout were considered as competitors, the planted trout fed mainly on mature insects (mostly Trichoptera and Odonata), and Diptera pupae (Table 1). In contrast, native brook trout ate three times more benthic organisms, and were the only ones to prey on northern redbelly dace. In Lake Cailloux and Lake Grignon, which contained white sucker and native brook trout as competitors, the planted brook trout fed on zooplankton (mostly Cladocera), benthic organisms (Ephemeroptera and Odonata larvae), and mature insects (Coleoptera and Odonata) in approximately similar proportions (Table 1). One exception was the individuals of the domestic strain in Lake Cailloux which fed on fish (northern redbelly dace) in a larger proportion. Compared to the planted brook trout, the native trout of these two lakes fed much less on Cladocera but more on fish (Table 1). Their diet also included benthic organisms (Odonata and Ephemeroptera larvae), mature insects and/or zooplankton

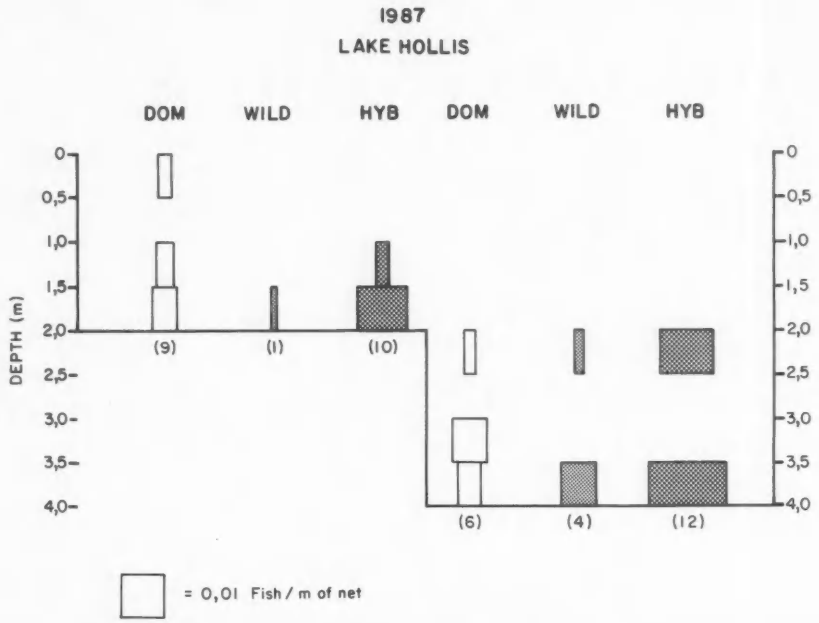

FIG. 2. Spatial distribution of brook trout in the littoral (2-m contour) and offshore (4-m contour) zones in Lake Hollis (brook trout lake) during the second summer after stocking (1987). DOM, domestic strain; WILD, wild strain, HYB, hybrid strain; NAT, native brook trout. Number of captures are in parentheses.

(Chaboridae larvae and Diptera pupae) (Table 1). Finally, individuals of the three strains planted in Lake Joe, which contained white sucker and native brook trout as competitors, fed mainly on benthic organisms (Ephemeroptera and Odonata larvae) and mature insects (Table 1). Here again, the individuals of the native strain differed from those of planted ones by having more Chaoboridae larvae, Diptera pupae, and fish in their diet (Table 1). In 1987, all planted trout found in Lake Cailloux, which contained white sucker and native brook trout as competitors, incorporated more fish (northern redbelly dace) in their diet than in 1986 (Table 2). Because of this change in their diet, the brook trout of the three planted strains had a diet similar to that of the native strain. In addition to fish, the trout of all four strains preyed mainly on benthic organisms (Ephemeroptera and Odonata larvae), and in certain cases on mature insects and/or Cladocera (Table 2).

We did not observe any general relationship between the fullness index and the strain of trout, or between the fullness index and the degree of competition (type of community; Table 1 and 2). The overall mean fullness index of all four strains of brook trout was $2.14 \pm 0.91 \times 10^{-3}$ g in most lakes in both years, but was $5.03 \pm 1.23 \times 10^{-3}$ g and $6.13 \pm 1.88 \times 10^{-3}$ in Lake Brochard (1986) and Lake Cailloux (1987), respectively.

Spatial Distribution

In Lake Deux Étapes, containing few competitors, individuals of the three planted strains were not distributed differently between littoral and offshore zones (Fig. 1). However, individuals of all strains were significantly more numerous in the lower part of the water column ($P < 0.05$), in the first year after planting (Fig. 1). Only one native brook trout was caught in 1986. In 1987, the domestic strain did not show any preference for zone, or part of the water column of the littoral zone (Fig. 2); however, in the offshore zone, this strain preferred the lower part of the water column ($P < 0.05$.) The hybrid strain preferred the offshore zone and the lower part of the water column in the offshore zone ($P < 0.05$; Fig. 2)). Finally, the wild strain had a significant preference for the offshore zone ($P < 0.05$), but not for a particular part of the water column (Fig. 2).

In lake Cailloux, containing white sucker and native brook trout, trout and suckers showed no significant preference for the offshore or littoral zones (Fig. 1). However, all strains of trout and suckers occurred mostly in the lower part of the water column ($P < 0.05$), although in three cases (domestic and wild strains in the offshore zone and wild strain in the littoral zone) differences were not significant (Fig. 1).

Reproductive Potential

During the first fall season following planting, only 3% of the wild strain females were mature (Table 3). In contrast, the females of the hybrid and domestic strains showed high percentages of maturity (84.6 and 91.4%, respectively). There were no significant differences between the females of these two strains with regard to GSI and egg diameter (Table 3). However, the fecundity of the domestic strain was significantly higher than that of the hybrid strain (ANCOVA, $F = 29.56$, $P < 0.001$; Table 3).

In 1987, the percentage of mature females in the wild strain had increased to 75%, whereas all the females of the domestic and hybrid strains were mature (Table 3). The mean GSI of the domestic strain females was significantly higher than the indices of the two other strains (ANOVA, $F = 16.48$, $P < 0.001$; Table 3). There were no significant differences among the three strains with regard to mean egg diameter. The domestic strain had a significantly higher fecundity than the hybrid strain, which in turn, had a significantly higher fecundity than the wild strain (ANCOVA, $F = 39.13$, $P < 0.001$; Table 3).

The males of the three planted strains showed a high percentage of maturity during the first fall season; 90.9, 94.4, and 93.3% for the domestic, hybrid and wild strains, respectively (Table 3). There were no significant differences in the mean GSI of the three strains. By the second fall season, all males caught were mature, and those of the hybrid strain showed the highest mean GSI (ANOVA, $F = 4.99$, $P < 0.05$; Table 3).

Discussion

The results of this study suggest that brook trout planted in lakes containing few competitors used the available food resources in the same way as native brook trout. The same was true in the presence of white sucker, but only in the second year after planting. Fraser (1981) also noted few differences in food habits between strains planted in the same lake. Furthermore, our results indicate that all fish (native and planted trout as well as white sucker) used the space in a similar manner, since they were mainly captured near the substrate in the littoral and offshore zones.

The results of the present study also suggest that planted brook trout underwent a feeding niche shift when living sympatrically with white sucker. In the absence of white sucker, the feeding habits of planted and native brook trout were comparable during the 2 yr of the study and there was no piscivory. When brook trout occurred sympatrically with white sucker, the native brook trout included fish (mainly *P. eos*) in their diet. During the first year after stocking, the feeding habits of planted brook trout differed from those of native trout in white sucker lakes, because planted trout did not prey on cyprinids. However, during the second summer, these planted trout ate fish and thus became similar to native brook trout in their diet. We conclude that such a feeding niche shift in planted brook trout is a phenotypic response to the presence of white sucker. This

TABLE 3. Weight, length, percent maturity, gonadosomatic index (GSI), fecundity, and egg diameter of individuals for the 2 yr after stocking, Lake Deux Étapes. DOM, domestic strain; HYB, hybrid strain; WILD, wild strain. Data are mean ± 1 SD in parentheses. Fecundity estimates were adjusted for a grand mean weight of 207.4 g in 1986, and of 605.9 g in 1987 using an ANCOVA (Sokal and Rohlf 1981).

	Females					
	1986			1987		
	DOM	HYB	WILD	DOM	HYB	WILD
Number captured	26	35	33	25	26	8
Weight(g)	226.8 (40.3)	195.8 (43.8)	106.2 (29.3)	642.8 (107.6)	634.7 (107.0)	430.9 (72.6)
Length (mm)	277.2 (16.5)	266.5 (19.6)	226.9 (19.9)	372.6 (21.3)	381.0 (18.2)	341.6 (17.1)
Mature females (%)	84.6	91.4	3.0	100.0	100.0	75.0
GSI[a]	5.8a (1.8)	5.1a (1.8)	—	17.6a (4.2)	12.8b (3.2)	10.1b (2.0)
Fecundity[b]	1585a	1242b	—	3283a	2680b	1746c
Egg diameter (mm)	1.97 (0.26)	2.02 (0.28)	—	3.63 (0.41)	3.45 (0.29)	3.76 (0.35)
	Males					
	1986			1987		
	DOM	HYB	WILD	DOM	HYB	WILD
Number captured	11	18	15	8	13	2
Weight(g)	248.0 (38.0	207.5 (44.7)	123.4 (23.7)	692.5 (98.5)	766.8 (104.8)	572.5 (68.0)
Length (mm)	285.5 (13.5)	271.7 (17.5)	231.9 (13.0)	395.1 (19.4)	409.9 (18.5)	385.5 (7.8)
Mature males (%)	90.9	94.4	93.3	100.0	100.0	100.0
GSI[a]	4.1a (1.2)	3.9a (1.0)	3.2a (1.1)	3.8a (0.9)	5.2b (1.9)	1.6a (1.5)

[a]Means with different letters are significantly different as determined by an ANOVA followed by a Student–Newman–Keuls multiple-range comparison test ($P<0.05$).

[b]Adjusted means with different letters are significantly different as determined by an ANCOVA followed by a Gabriel approximate comparison interval ($P<0.05$; Sokal and Rohlf 1981).

niche shift is, however, different from that observed in self-sustaining brook trout populations (i.e. in lakes where brook trout are able to sustain fishing exploitation without any fish stocking; see Magnan 1988). S. Tremblay and P. Magnan (unpubl. data) observed that such a brook trout population shifted its feeding habits from zoobenthos to zooplankton rather than to fish when living in sympatry with white sucker. The feeding niche shift was also confirmed in 12 other lakes of the same area (Magnan 1988). The white sucker fed mainly on zoobenthos in these lakes (Magnan 1988; S. Tremblay and P. Magnan, unpubl. data). The observed differences in the niche shift of self- and non-self-sustaining populations of brook trout to sport fishing may be related to inherent differences in the characteristics of the lakes that contain these populations. These differences may also be related to the genetic backgrounds of the fish, which either reproduce naturally or which are planted from stocks originating from elsewhere. However, because the three strains used in the present study originated from completely different areas (Lachance and Magnan 1990), and because their feeding habits were similar during the 2 yr of the study, it is unlikely that differences in genetic backgrounds contributed to the observed differences in the niche shift of self- and non-self-sustaining populations of brook trout.

Size, as well as percentage of body lipid (not age or growth rate) are the factors determining sexual maturity in brook trout (McCormick and Naiman 1984). According to McCormick and Naiman (1984), females should reach a greater size than males before they become mature. The results of this study agree with this conclusion; sexual maturity was related to the size of individuals, regardless of the strain, and males became mature before females. The lower percent of mature wild females during the 2 yr of the study could be explained by their smaller size at capture which followed from their smaller size at planting (Lachance and Magnan 1990). However, over a period of 2 yr following stocking, the wild and hybrid strains had better rates of recovery and yield than the domestic strains, even though individuals of domestic strains had higher (or similar) weights (Lachance and Magnan 1990). Therefore, the wild and hybrid strains may offer a better potential for reproduction, after stocking, than the domestic strain. The selection of wild or hybrid strains for stocking should thus be decided on the basis of whether or not to preserve the indigeneous genetic pool of brook trout in the lakes to be stocked.

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Experimental Infection of Rainbow Trout, *Oncorhynchus mykiss*, with *Contracaecum osculatum* (Rudolphi, 1802) and *Pseudoterranova decipiens* (Krabbe, 1878) (Nematoda: Ascaridoidea)

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The fate of freshly hatched larvae of the marine ascaridoid nematodes *Contracaecum osculatum* and *Pseudoterranova decipiens* (from grey seals, *Halichoerus grypus*, from Scotland and the Canadian Atlantic) was investigated following oral or intraperitoneal introduction into rainbow trout, *Oncorhynchus mykiss*, maintained at 7–13°C in fresh water. Neither species appeared to survive for long in the trout alimentary tract following oral introduction; a few larvae were found alive after 2 d but none after 21 d. Intraperitoneally, *P. decipiens* did not survive beyond 21 d, but some *C. osculatum* exsheathed and developed over several months to lengths over 13 mm, and morphologically and morphometrically resembled third-stage larvae from naturally infected whiting, *Merlangius merlangus*, from the northern North Sea; no moult was detected. Thus, freshly hatched free-living larvae of *C. osculatum* are able to develop directly to the third stage in the body cavity of a fish without earlier passage through a crustacean or other invertebrate host.

Le destin des larves fraîchement écloses d'Ascaridoides marins *Contracaecum osculatum* et *Pseudoterranova decipiens* (du phoque gris, *Halichoerus grypus*, d'Ecosse et de l'Atlantique canadien) a été étudié après sa pénétration par voie orale ou intrapéritonéale dans la truite arc-en-ciel, *Oncorhynchus mykiss*, placée en eau douce à une température de 7 à 13°C. Aucune des espèces n'a semblé survivre longtemps dans le tube digestif de la truite après son ingestion par voie orale; quelques larves ont été retrouvées vivantes après 2 j, mais aucune après 21 j. *Pseudoterranova decipiens*, ingéré par voie intrapéritonéale, n'a pas survécu au-delà de 21 j, mais certains *C. osculatum* ont mué et se sont développés pendant plusieurs mois pour atteindre une longueur de plus de 13 mm, et sur le plan morphologique et morphométrique, ils ressemblaient aux larves du troisième stade provenant de merlans, *Merlangius merlangus*, naturellement infestés fréquentant la partie nord de la Mer du Nord; aucune mue n'a été observée. Donc, des larves libres fraîchement écloses de *C. osculatum*, peuvent se développer directement au troisième stade dans la cavité générale d'un poisson sans qu'il y ait passage par un hôte de type crustacé ou d'un autre invertébré hôte.

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Larvae of certain marine ascaridoid nematodes ("sealworms" and "whaleworms") occur in the flesh of commercially important fish where they are aesthetically unattractive to consumers and have public health significance (Malouf 1986). In 1984 in Atlantic Canada alone the costs to the fishing industry of detecting and removing the larvae and of reduction in the value of the final fish products were estimated at $30 million (Malouf 1986).

With regard to nematodes in general, six stages (namely egg, four larval stages, and adult) form the basis for a great variety of life history patterns that have been recognized among free-living and parasitic forms, both terrestrial and aquatic (Chitwood 1974). First-, second-, third- and fourth-stage larvae are conventionally referred to as L1, L2, L3, and L4, respectively. Four moults are associated with development from L1 through L2, L3, and L4 to the adult stage. First and second-stage larvae (i.e. L1 and L2) may retain the cuticle into the succeeding stage as a sheath. Such larvae normally shed the sheath, through the process of exsheathment, before the next moult takes place. With respect to early stages in the life cycle, one or two moults

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may occur within the egg. If one moult takes place, it is an ensheathed L2 that hatches, whereas if two moults occur within the egg, it is an ensheathed L3 that hatches. The stage infective to the final host, in which the adults are found, is usually the L3.

The final hosts of marine ascaridoid nematodes are marine mammals (seals and cetaceans). Our knowledge of the life history patterns of marine ascaridoids is deficient, especially regarding the early stages, with uncertainty surrounding, for example, the number of moults which take place within the egg of the various species, and the "paratenic" or "intermediate" host status of the invertebrates (e.g. crustaceans) and vertebrates (e.g. fish) which have been found naturally infected with larvae or have been experimentally infected. Smith (1983) and McClelland (1990), among others, have reviewed a confusing parasitological literature on these aspects.

As part of investigations into the early stages of development of marine ascaridoids (see also Burt et al. 1990), two experiments (A and B) were conducted. Freshly hatched larvae of "sealworm" *Pseudoterranova decipiens* (Krabbe, 1878) and of *Contracaecum osculatum* (Rudolphi, 1802), a related marine ascaridoid the final hosts of which are also seals, were introduced orally and intraperitoneally (=i.p.) into rainbow trout, *Oncorhynchus mykiss* (formerly known as *Salmo gairdneri*). While rainbow trout are "unnatural" hosts of larval marine ascaridoids, they have the advantage of easy maintenance in freshwater aquaria, and there are some records of larvae of the marine ascaridoid *Anisakis* ("whaleworm") in wild and cultured trout in fresh water (Wootten and Smith 1975).

Materials and Methods

For experiment A (i.p. infection only), the uterus was removed from each of 20 gravid *C. osculatum* harvested from grey seals, *Halichoerus grypus*, of Scottish east coast origin (northern North Sea). The release of eggs from the uteri and their antibiotic-fungicide treatment and incubation in sterile seawater at 15°C followed McClelland and Ronald (1974a). Freshly hatched larvae were artificially exsheathed in 0.05% sodium hypochlorite (Davey 1969). Twenty-two rainbow trout were anaesthetised with benzocaine, and 1.0 mL from a stock of sterile phosphate-buffered saline (PBS) containing several thousand artificially exsheathed larvae in stirred suspension was injected into the body cavity through the right flank, each fish receiving several hundred larvae. The fish recovered and were maintained in fresh water.

For experiment B (i.p. and oral infection), uteri were removed from 50 gravid *P. decipiens* from grey seals caught in the Canadian Atlantic (Port Hood, Gulf of St Lawrence) and transported, chilled on ice, to Aberdeen. It was not recognised until the eggs had hatched that at least one uterus was *C. osculatum* rather than *P. decipiens*; excluding the sheath, hatched larvae of *P. decipiens* are about 0.20 mm long whilst those of *C. osculatum* are about 0.40 mm long (Brattey 1990). No attempt was made to exsheath *P. decipiens* larvae before use in either oral or i.p. infection; McClelland and Ronald (1974a) found no suitable technique for achieving artificial exsheathment in *P. decipiens*. Several hundred ensheathed larvae, representing a mixed infection dominated by *P. decipiens*, were injected i.p. into 10 trout using the method described for experiment A. A second batch of 10 trout was orally infected by feeding them on 1.0 cm lengths of chopped earthworm injected with 1.0 mL of PBS containing several hundred ensheathed larvae of the mixed infection.

TABLE 1. *Contracaecum osculatum* in rainbow trout (experiments A and B): numbers and condition at various intervals following intraperitoneal introduction as freshly hatched artificially exsheathed (experiment A) or ensheathed (experiment B) larvae. All larvae recovered were alive.

Day (experiment A/B) post-infection	Number of fish	Number of *C. osculatum* larvae per fish
2 (A)	1	Several hundred
2 (B)	1	Several hundred (ensheathed)
6 (A)	1	0
10 (A)	1	0
15 (A)	2	0, 0
20 (A)	1	0
21 (B)	1	1 (fully exsheathed)[a]
30 (A)	2	2, 0
42 (B)	1	0
60 (A)	2	6, 0
77 (B)	1	0
120 (A)	1	37
150 (A)	2	1, 12
180 (A)	3	81, 72, 0
189 (B)	1	5
210 (A)	3	48, 32, 0
255 (B)	4[b]	10, 5, 0, 0
270 (A)	3	48, 16, 0

[a]In experiment B, all larvae from day 21 onwards were fully exsheathed and had grown (see also Table 2).
[b]A tenth fish died in experiment B and was not examined.

The 42 trout used in the two experiments originated from Scottish commercial freshwater farms as yearlings about 10 cm long. The last ones to be killed and examined some 8 mo later had grown to about 30 cm long. Before and during the experiments the water temperature varied between 7 and 13°C, and the trout were maintained on dried pelleted food. The trout were killed by anaesthetic overdose at various intervals after infection (Tables 1 and 2).

At post-mortem examination, the body cavity of each fish was opened mid-ventrally, and the alimentary tract, associated organs, and mesenteries were removed to a dish of PBS where they were teased apart and searched for worms with the aid of a stereomicroscope. The gastrointestinal tract was opened and PBS flushings of the lumen were searched for worms. The musculature was digested at 37°C in a pepsin-HC1 solution and sieved for worms following Smith and Wootten (1975). For fish killed early in each experiment, when the host biomass was large relative to the small size of worms, the viscera were immersed in PBS and warmed for 1 hr at 37°C to make larvae more active and easier to find. Both the viscera and flesh of fish killed on day 42 and subsequently were digested and sieved.

All worms recovered were fixed in hot glycerin-ethanol for subsequent microscopical examination. Where possible, 10 *C. osculatum* larvae were measured at each time interval post-infection in experiment A; in experiment B, all available larvae were measured (Tables 1 and 2). Table 2 also includes, for comparison, measurements of 36 *C. osculatum* L3 from the liver parenchyma of whiting, *Merlangius merlangus*, caught commercially in the northern North Sea (36 miles north east by north of Fraserburgh, Scotland).

TABLE 2. Measurements (in mm) of 69 *Contracaecum osculatum* larvae recovered from days 21 to 270 from 15 intraperitoneally infected rainbow trout (experiments A and B), and comparison with 36 third-stage larvae (L3) from naturally infected whiting.

Day (experiment A/B) post-infection	Number measured	Mean (range) length					
		Whole body	Preventriculus	Intestinal caecum	Ventricular appendix	Mid-body width	Caecum/ appendix
21 (B)	1	0.56 (—)	—	—	—	—	—
30 (A)	2	0.87 (0.80, 0.94)	—	—	—	—	—
60 (A)	6	2.48 (2.20–2.66)	—	—	—	—	—
120 (A)	10	3.19 (2.50–6.54)	0.66 (0.53–0.91)	0.42 (0.23–0.53)	0.60 (0.46–0.91)	0.18 (0.10–0.30)	0.70 (0.50–0.80)
150 (A)	10	4.92 (2.89–6.73)	0.75 (0.53–0.91)	0.41 (0.27–0.53)	0.77 (0.53–1.06)	0.22 (0.14–0.30)	0.53 (0.40–0.70)
180 (A)	10	5.95 (3.50–8.28)	0.82 (0.61–1.06)	0.51 (0.39–0.61)	0.81 (0.53–0.99)	0.26 (0.19–0.31)	0.64 (0.50–0.80)
189 (B)	3[a]	7.29 (6.56–8.14)	0.87 (0.75–0.96)	0.47 (0.38–0.57)	0.78 (0.66–0.85)	0.27 (0.23–0.31)	0.63 (0.45–0.86)
255 (B)	13[b]	8.14 (6.80–10.70)	0.99 (0.84–1.23)	0.53 (0.42–0.73)	0.77 (0.62–0.91)	0.25 (0.23–0.30)	0.70 (0.52–0.88)
270 (A)	10	10.19 (6.99–13.60)	1.18 (0.91–1.37)	0.69 (0.53–0.84)	1.11 (0.76–1.29)	0.34 (0.23–0.42)	0.64 (0.50–0.80)
L3 from whiting	36	17.40 (8.50–26.30)	1.37 (0.96–2.01)	0.92 (0.50–1.39)	1.34 (0.88–2.41)	0.53 (0.33–0.73)	0.69 (0.51–1.50)

[a]Two other (damaged) specimens were 4.23 and 5.19 mm long.
[b]Two other (damaged) specimens were 6.33 and 7.21 mm long.

Results

Neither *C. osculatum* nor *P. decipiens* larvae were recovered from the musculature of rainbow trout, following either oral or i.p. introduction.

When given orally (experiment B), very few larvae of either *C. osculatum* or *P. decipiens* were found alive in the gastrointestinal tract lumen after 2 d; at 21 d there were no *P. decipiens* larvae and only one (dead) *C. osculatum* larva.

A few *P. decipiens* larvae introduced i.p. (experiment B) exsheathed in the body cavity but survived only for 21 d. In sharp contrast, some *C. osculatum* larvae injected i.p. (experiments A and B) survived in the body cavity for up to 270 d, and grew considerably (Tables 1 and 2). Those *C. osculatum* injected i.p. as ensheathed larvae (i.e. in experiment B) had naturally exsheathed by day 21 (Table 1). The older larvae morphologically and morphometrically fitted the description of *C. osculatum* L3 given by, for example, Berland (1961) and Smith and Wootten (1984). Moreover, Elarifi's (1981) morphometric regression analyses for the length or position of various body structures for both *C. osculatum* L3 from naturally infected whiting and for larvae reared in vivo in rainbow trout (Table 2) showed a good fit to larvae reared in vitro by McClelland and Ronald (1974b).

After day 30, *C. osculatum* larvae were found in capsules of host origin within the body cavity of their fish hosts; they were occasionally located near the site of original injection or on the peritoneum, but occurred mostly on mesenteries associated with the pyloric caeca.

Despite the introduction i.p. of several hundred *C. osculatum* larvae into each of 22 trout in experiment A, apparently no worms became established in 10 fish (Table 1).

Discussion

The present results demonstrate that freshly hatched free-living larvae of *C. osculatum* are able to exsheath and develop directly to third stage in the body cavity of a fish without earlier passage through a crustacean or other invertebrate host. During growth to over 13 mm in length over a period of several months, these larvae developed and came to resemble L3 of *C. osculatum* from naturally infected whiting. No moult was detected but it could have been missed as fish were not examined at close intervals following day 2. Artificial exsheathment of *C. osculatum* larvae was readily achieved for experiment A, but experiment B demonstrated this to be an unnecessary procedure because ensheathed larvae fared just as well in the body cavity.

The fate of ensheathed *P. decipiens* injected i.p. into a teleost does not appear to have been investigated previously. The few survivors in the body cavity of trout at 21 d had exsheathed but, in contrast to *C. osculatum*, they had not grown and none was found at 42 d or thereafter.

Following oral administration, the early disappearance of both *C. osculatum* and *P. decipiens* larvae from the gastrointestinal tract of rainbow trout in fresh water agrees with Myers' (1960) finding for *P. decipiens* in teleosts in seawater. Perhaps freshly hatched larvae of these species cannot survive the physicochemical environment of the trout gastrointestinal tract and (or) cannot penetrate the gut wall to reach the body cavity.

There are no ready explanations for the absence of *C. osculatum* from the body cavity of some trout injected i.p.; possibly there was failure to introduce a sufficient number of larvae into these fish during the i.p. injection procedure, or some fish were refractory to infection.

As was expressed in the introduction to this paper, there is uncertainty regarding the number of moults which take place in the egg of the various marine ascaridoid nematode species.

Smith (1983) argued that only one moult occurs in the egg of "whaleworm" *Anisakis simplex* B, i.e. an ensheathed L2 hatches, the second moult (to L3) occurring in crustaceans (predominantly euphausiids). However, McClelland (1990) reviewed evidence which suggests that in *P. decipiens* it is an ensheathed L3 that hatches. The present results afford no conclusion regarding this aspect for either *P. decipiens* or *C. osculatum*. Much work remains to be done on the life history of marine ascaridoids.

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Microhabitat Partitioning among Stream-Dwelling Juvenile Coho Salmon, *Oncorhynchus kisutch*, and Dolly Varden, *Salvelinus malma*

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Dolloff, C. A., and G. H. Reeves. 1990. Microhabitat partitioning among stream-dwelling juvenile coho salmon, *Oncorhynchus kisutch*, and Dolly Varden, *Salvelinus malma*. Can. J. Fish. Aquat. Sci. 47: 2297–2306.

Microhabitat use and partitioning among age 0+ and 1+ coho salmon, *Oncorhynchus kisutch*, and age 0+, 1+ and 2+ Dolly Varden, *Salvelinus malma*, was studied in small (<2 m wide), natural streams on Prince of Wales Island, Alaska, and in laboratory stream channels. Coho salmon occupied midwater positions that they defended from other fish. Dolly Varden were more closely associated with the stream bottom and were seldom territorial. For each species, the depth of water, depth of focal point, and distance to nearest fish increased with fish size, whereas the distance to nearest cover decreased as fish size increased. Most fish selected focal point velocities between 0.0–9.0 $cm \cdot s^{-1}$. Woody debris was the most frequently used cover type and most fish occurred over gravel substrates ranging from 2–100 mm particle diameter. Habitat use by each species in the laboratory was similar to the pattern observed in the field. Each species occupied similar habitats both when alone and when the other species was present. Although habitat use by juveniles of coho salmon and Dolly Varden overlapped among several key parameters, each species primarily exploited resources not readily available to or selected by the other in the natural streams we studied.

L'utilisation du microhabitat et son fractionnement entre les saumons cohos, *Oncorhynchus kisutch*, d'âge 0+ et 1+, et les Dolly Varden, *Salvelinus malma*, d'âge 0+, 1+ et 2+ ont été étudiés dans de petits (<2 m de largeur) cours d'eau naturels de l'île Prince-de-Galles (Alaska), et dans des chenaux d'essais. Les saumons cohos fréquentaient les eaux semi-pélagiques q'uils défendaient contre les autres poissons. Les Dolly Varden étaient plus étroitement associées au fond des cours d'eau et elles étaient rarement territoriales. Pour chaque espèce, la profondeur de l'eau, la profondeur du point focal, et la distance jusqu'au poisson le plus proche augmentaient avec la taille du poisson, tandis que la distance jusqu'au couvert le plus proche diminuait à mesure que la taille du poisson augmentait. La plupart des poissons choisissaient des vélocités au point focal entre 0,0 et 0,9 $cm \cdot s^{-1}$. Des débris de bois constituaient le type de couvert le plus souvent utilisé et la plupart des poissons se trouvaient au-dessus de substrats de gravier dont le diamètre des particules était compris entre 2 et 100 mm. En laboratoire, l'utilisation de l'habitat par chaque espèce était semblable au profil observé dans les cours d'eau. Chaque espèce occupait des habitats semblables, lorsqu'elles étaient seuls et en présence d'autres espèces. Même s'il y a chevauchement de plusieurs paramètres clés de l'utilisation de l'habitat par les juvéniles de saumon coho et de Dolly Varden, chaque espèce exploitait principalement les ressources qui ne sont pas accessibles à l'autre espèce ou qu'elle ne choissait pas dans les cours d'eau naturels étudiés.

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Juvenile coho salmon, *Oncorhynchus kisutch*, and Dolly Varden, *Salvelinus malma*, occupy similar habitats in small streams throughout southeast Alaska. Both species typically remain in freshwater for two or more years which results in the formation of discrete age-(size) classes (Crone and Bond 1976). Coexistence among the members of these species-age class groups depends upon partitioning scarce resources such as microhabitat space and food. Under the assumptions of resource partitioning, members of each group would use a unique subset from the available resource pool. Differences in resource use among similar species in the same habitat are known to arise from both interactive and selective processes (Brian 1956), and have been described for other assemblages of salmonids (Nilsson 1967).

Interactive segregation occurs when differences in patterns of resource use between groups are increased by the interactions (i.e. competition, predation) among group members. Interactive segregation has been shown to occur between coho salmon and steelhead trout, *Oncorhynchus mykiss* (Hartman 1965), and

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TABLE 1. Cover categories utilized in the microhabitats of juvenile coho salmon and Dolly Varden in four Southeast Alaska streams.

Cover category	Description
Cobble	Rocks of approximately 100–300 mm diameter on the streambed.
Boulder	Rocks > cobble with space underneath suitable for hiding.
Log	Wood debris approximately 10 cm to 1 m in diameter.
Branches	Wood debris 10 cm anchored to the streambed.
Debris plus overhead vegetation	Stream bank vegetation intertwined with branches.
Fine debris	Loose collection of wood and other debris.
Debris plus undercut bank	Branches supporting or extending from an earth bank.
Overhead vegetation	Trees or other vegetation extending over the water surface.
Aquatic vegetation	Primarily leaves of *Symplocarpus* sp. (Skunk cabbage).
Undercut bank	Overhanging earth bank carved by water current.
Overhead vegetation plus undercut bank	Vegetation and earth bank.

TABLE 2. Substrate at the focal points of juvenile coho salmon and Dolly Varden in four southeast Alaska streams.

Substrate category	Description
Silt	Flocculent particles 0.06 mm.
Sand	Particle size > silt and ≤ 2 mm.
Small gravel	Particle size > sand and ≤ 12 mm.
Medium gravel	Particle size > small gravel and ≤ 100 mm.
Large gravel	Particle size > medium gravel and ≤ 300 mm.
Rock	Particle size > large gravel but not embedded.
Bedrock	Shelf of embedded rock.
Clay	Smooth earth surface, no rock.
Fine debris	Wood and vegetative debris particles.
Log	Wood debris ≥ 10 cm diameter.

between coho salmon and cutthroat trout, *O. clarki* (Glova 1978).

Contrasting with the interactive mode of segregation, differences in resource use can also arise from differences in instinctive behavior or body morphology; each species or group uses resources not "selected" by the other, resulting in neutral interactions among groups. Schutz and Northcote (1972) concluded that adult cutthroat trout and Dolly Varden in a British Columbia lake were segragated selectively by innate morphological and behavioral differences. A more recent study of resource use by lake-dwelling Dolly Varden and cutthroat trout, however, suggests that the vertical distribution of Dolly Varden and their consumption of zoobenthos are influenced by the more aggressive cutthroat trout (Hindar et al. 1988).

In this paper we describe the patterns of microhabitat use among members of coho salmon — Dolly Varden assemblages in natural and laboratory streams and examine possible mechanisms for coexistance in a series of laboratory manipulations.

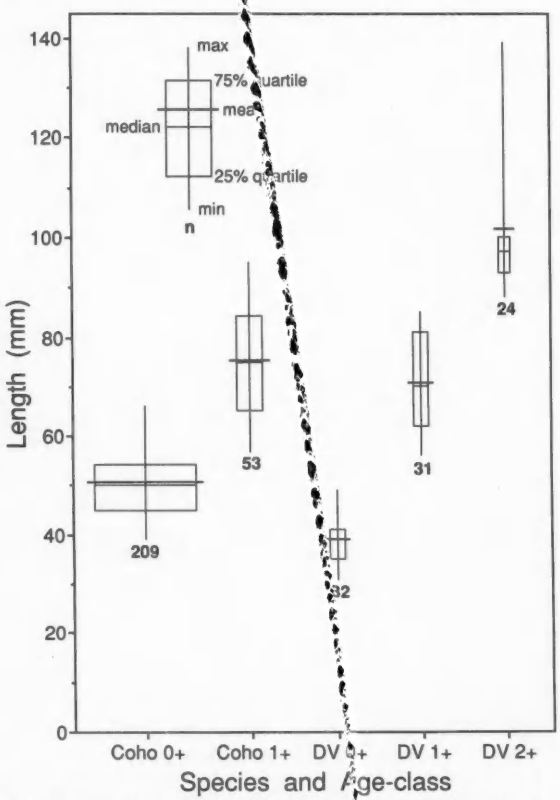

FIG. 1. Box plots of total lengths of juvenile coho salmon and Dolly Varden in four southeast Alaska streams during July and August, 1981. Sample size (n) is proportional to box width.

Methods

Field

Populations of coho salmon and Dolly Varden were observed from the banks of four small (<2 m wide) tributary streams in the Staney Creek drainage on Prince of Wales Island, Alaska. Three of these streams were located in recent (5–10 yr old) clearcut forest whereas the fourth stream flowed through old-growth coastal rain forest. Stream temperatures were measured with maximum-minimum thermometers and ranged from 8 to 18°C, and discharge ranged from about 1 to 150 $L \cdot s^{-1}$ during

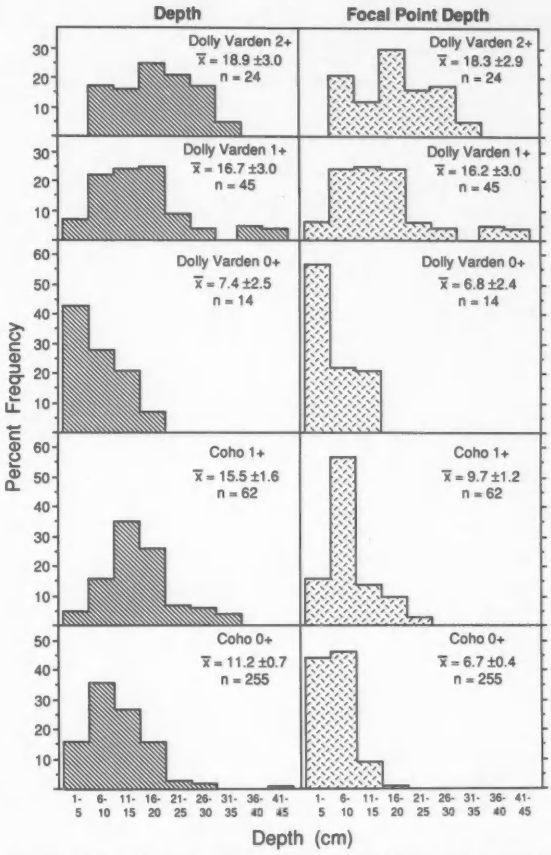

FIG. 2. Frequency distributions, means, and 95% confidence intervals for water depths and focal point depths occupied by coho salmon and Dolly Varden.

the summer. All four streams contained comparable loads of woody debris input both by natural processes and as a byproduct of logging. Population densities ($N \cdot m^{-2}$) during the period of observation ranged from 0.8–1.9 for coho salmon and from 0.2–0.7 for Dolly Varden (Dolloff 1983). Steelhead or cutthroat trout were occasionally seen but were not present in the sections chosen for observation.

Microhabitat observations were conducted during a 6-wk period in July and August 1981 on rain-free, windless days between 09:00 and 15:00. Observation sites were discrete stream areas bounded by debris dams, shallow water riffles, or other natural features. Observers remained as inconspicuous as possible for the duration of the observation; fish disturbed by the observer's approach appeared to resume normal activity after about 10 min. The observer watched until satisfied that all fish occupying focal points had been identified and their locations recorded on a field sketch. This tyically required 1–3 h.

Painted and numbered markers corresponding to the different species and size-(age) classes present were placed on the stream bottom under the focal point of each fish. Fish not having specific focal points also were noted and markers were placed at the locations they most frequently occupied. Following an additional 10–20 min observation period to verify and correct the placement of markers, focal point characteristics including water depth and velocity (measured with an electronic flowmeter), depth in the water column, distance to and type of nearest cover (Table 1), and substrate type and size (Table 2), were measured. Because it was not always possible to associate a unique cover type with a particular fish, three composite cover categories were created and treated as distinct cover types (Table 1). The distance to and species of nearest fish were recorded. The site was then photographed and its dimensions and essential habitat features recorded on the field sketch.

Where conditions permitted, fish were captured with a backpack electrofisher and identified by species, measured to the nearest 1.0 mm fork length (FL), and weighed to the nearest 0.1 g. Scales were taken from a subsample of captured fish to verify age-classes. Although measurable growth occurred during the 6-wk observation period (Dolloff 1983), size differences remained sufficient to distinguish between age-classes of both species.

Laboratory

Laboratory tests were conducted in two oval shaped, artificial streams (Reeves et al. 1983) located at the USDA Forest Service Pacific Northwest Experiment Station in Corvallis, Oregon from October 1987 to February 1988. Channels were 0.76 m wide, 0.61 m deep, and 18.40 m in circumference. The streams were stacked one above the other, and black plastic curtains shielded fish from outside disturbances. Water temperature, current velocity, and photoperiod were regulated by separate systems in each channel. Daily water temperature varied from 8°C in the morning to 12°C in the evening, as was typically observed in the field. Current velocity ranged from 0–10 $m \cdot s^{-1}$. The photoperiod of 15L:9D included a 1.5 h "morning" where light increased gradually from zero to full intensity, and a corresponding 1.5 h "evening" where light decreased to zero.

Each channel had four pools (25 cm deep) with sand substrate and four riffles (12 cm deep) with gravel and cobbles. In two of the pools, overhanging black Plexiglas provided cover. Cover in each of the two remaining pools consisted of one piece of lumber 60 cm long and 10 cm^2 in cross section that was studied with numerous 30 cm-long dowels. The dowels ranged from 0.60–1.25 cm diameter and were configured to represent natural woody debris. Larger cobbles provided cover in the riffles.

For each trial, streams were sterilized with a bleach solution, rinsed, and filled with Corvallis city water at least 5 d before fish were introduced. Water was continually filtered through a sand bed and sterilized by ultraviolet light.

Juvenile coho salmon and Dolly Varden were captured in October and November 1987 in Maybeso Creek on Prince of Wales Island, Alaska using baited minnow traps. Captured fish were checked for obvious signs of disease or injury and held temporarily in live cages before being flown in cooled, aerated containers from Alaska to Oregon. The fish arrived in Corvallis within 10 h of removal from the stream and were held in tanks filled with well water. Before they were introduced to the laboratory streams, the fish were again observed for injury or abnormal behavior and were fed a mixed diet of frozen brine shrimp (*Artemia* sp.) and Oregon Moist Pellets. No injuries or abnormalities associated with travel were observed.

Individual fish were measured to the nearest 1.0 mm FL and weighed to the nearest 0.1 g before being placed in the chan-

TABLE 3. Kolmogorov Smirnov two tailed test p-value for differences in use of five microhabitat parameters between groups of coho salmon and Dolly Varden in four southeast Alaska streams.

Species and age-class	Depth		Distance to nearest		
	Water	Focal point	Cover	Fish	Conspecific
Coho salmon 0+ and 1+	0.001	0.001	0.198	0.010	0.001
Coho salmon 0+, Dolly Varden 0+	0.081	0.889	0.393	0.218	0.003
Coho salmon 0+, Dolly Varden 1+	0.007	0.001	0.001	0.012	0.020
Coho salmon 0+, Dolly Varden 2+	0.001	0.001	0.001	0.270	0.293
Coho salmon 1+, Dolly Varden 0+	0.003	0.043	0.697	0.005	0.609
Coho salmon 1+, Dolly Varden 1+	0.836	0.001	0.001	0.001	0.001
Coho salmon 1+, Dolly Varden 2+	0.024	0.001	0.001	0.007	0.003
Dolly Varden 0+ and 1+	0.014	0.005	0.057	0.882	0.051
Dolly Varden 0+ and 2+	0.002	0.001	0.001	0.988	0.164
Dolly Varden 1+ and 2+	0.055	0.347	0.052	0.912	0.308

nels. Mean size ranged from 57.9–72.6 mm FL and 2.2–4.5 g for age 0+ and 83.4–91.2 mm FL and 6.4–8.2 g for age 1+ coho salmon. Dolly Varden ranged from 71.6–97.5 mm FL and 3.7–8.6 g. Eight age 0+ and four age 1+ coho salmon and 12 Dolly Varden were used for all trials. A total of six trials was run; two with each species alone and two together. Fish were introduced 48 h before the start of observations. Upstream and downstream traps were opened after 24 h to allow fish that were unable to occupy sites to leave the channel. Traps remained open for the duration of the 7 d observation period.

Fish were fed a daily ration of frozen brine shrimp equal to 15% of the wet weight of fish at the start of the trial. Half of the ration was offered in the morning, and 25% was offered at midday and again in the evening. The food was delivered through a perforated 2.5 cm diameter pipe that ran in a zig-zag pattern across the entire area available to fish (Reeves and Everest 1988). About 2.3 g of live *Tubifex* sp. worms per fish were equally distributed in pools on the third or fourth night of each trial to provide a source of benthic prey.

Observations were made before the midday feeding. The number and location where each fish spent the most time and the number of inter- and intraspecific interactions were recorded in each pool and riffle during successive 3 min observation periods. Grids superimposed on the side of the channels and on the substrate were used to estimate the height above substrate and distance to cover. The sequence of observation was randomly determined for each trial. Six trials were run: two each for coho salmon and Dolly Varden alone and two together for a total observation period of 1,008 min.

Mann–Whitney and Kolmogorov–Smirnov two-sample tests (Conover 1971) and t-tests were used to detect differences in microhabitat variables between all species — age-class groups. Stepwise discriminant analysis was used to identify interactions between fish groups and to determine the relative importance of resource categories in distinguishing microhabitat use by species and age-class (Keclka 1975).

Results

Field

We found only minor differences in values of microhabitat variables among streams and therefore pooled data from all microhabitat sites for subsequent analyses. A comparison of total lengths for a sample of the juvenile coho salmon and Dolly Varden illustrates the minimal overlap in size among both species and age-classes within species (Fig. 1). The slight overlap between coho salmon age-classes reflects the relatively fast growth of age 0+ fish. By the end of the observation period, these fish were as large as the age 1+ fish had been only 6 wk earlier. Fish size within individual observation sites did not overlap.

Water Depth and Depth of Focal Point

Both age classes of coho salmon used specific midwater locations with age 1+ coho salmon occupying sites in deeper water (Fig. 2). Age 0+ Dolly Varden did not occupy specific focal points but tended to remain within confined, shallow water areas where they typically were observed on or near the bottom. Older Dolly Varden usually occupied focal points located in the deepest available water on or near the streambed. These fish would occasionally forage away from their focal points but would return to them periodically or when disturbed.

With few exceptions, the use of water depth (D) and depth of focal point (FPD) differed among the species-age class groups (Table 3). Yearling coho salmon and Dolly Varden were observed at similar Ds but Dolly Varden had deeper FPDs. Age 0+ coho salmon and Dolly Varden apparently occurred at similar Ds and FPDs; however, the shallow focal point depths assigned to the Dolly Varden resulted from the shallow depth of water in which they were observed. Age 1+ and 2+ Dolly Varden used similar Ds and FPDs.

Distance to Nearest Cover and Nearest Fish

The distance from focal points to nearest cover (DTNC) decreased for both species with increasing age (Fig. 3). The affinity for cover was most apparent in yearling and age 2+ Dolly Varden of which 53 and 88%, respectively, were found in cover (DTNC = 0). Differences between species were more pronounced than differences within species for this variable (Table 3).

The distance to nearest fish (DTNF) was used as a generalized indicator of agonistic tendencies. Tolerance towards other fish as measured by the DTNF was greatest for subyearling Dolly Varden and least for yearling coho salmon (Fig. 3). For coho salmon, the DTNF increased with age. The DTNF and age were not related for Dolly Varden. As was the case for DTNC, the differences in DTNF were more pronounced between species than among age-classes (Table 3).

The distance to nearest conspecific fish (DTNCF) was measured to obtain an indication of the degree of territorial behavior exhibited by a fish toward other fish of the same species and age-class (Fig. 3). For all groups, DTNCF was greater than

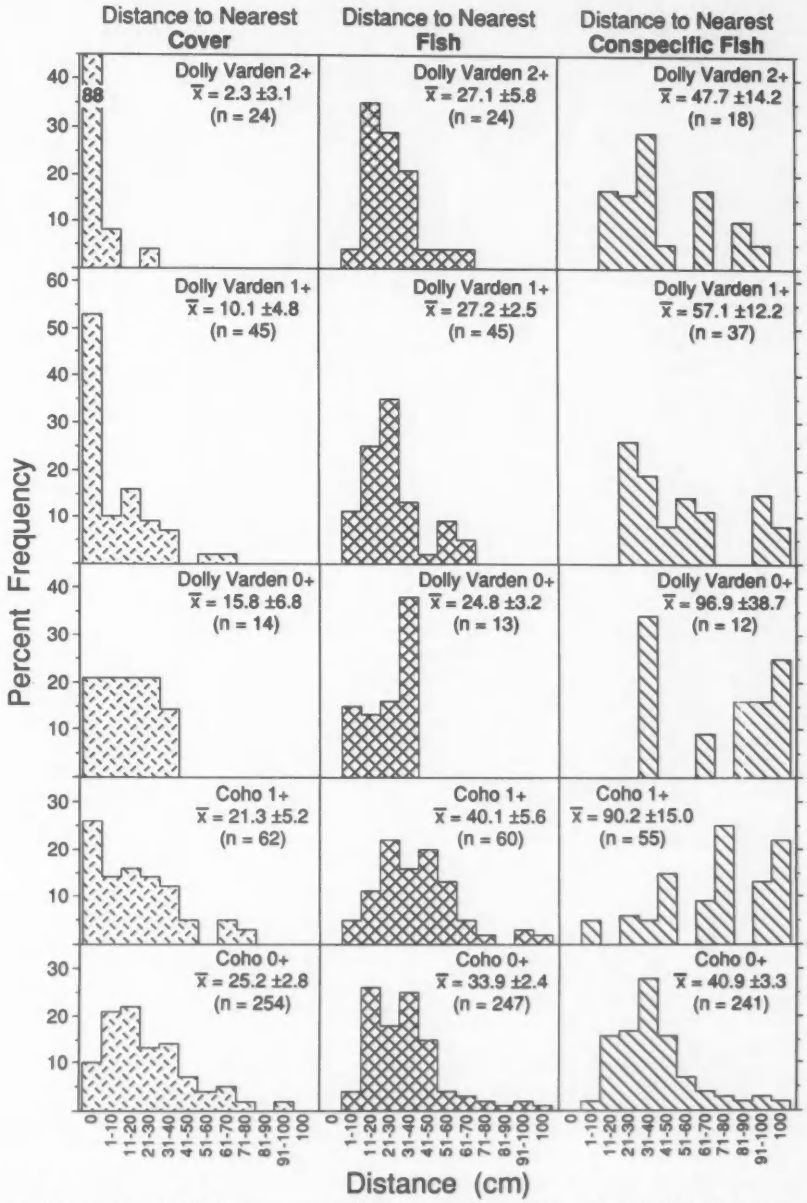

FIG. 3. Frequency distributions, means, and 95% C.I. for distance to nearest cover, distance to nearest fish, and distance to nearest conspecific fish for coho salmon and Dolly Varden.

DTNF, suggesting greater aggression towards conspecifics. Because of complications introduced by the local abundance of conspecific fish and the physical characteristics of a site, however, the DTNCF did not accurately reflect territoriality. The distribution of values for the DTNCF maintained by yearling coho salmon suggests that while these fish were highly territorial, at least a few tolerated conspecifics at close range. For yearling coho salmon, the DTNCF may have been influenced both by the local abundance of conspecifics and by the degree of visual isolation. At several sites, woody debris or streambed topography visually isolated adjacent fish, thereby allowing them to coexist despite their proximity. Age 0+ Dolly Varden appeared to be highly territorial because their average DTNCF exceeded all others. However, the distribution of values for the DTNCF was related to the relative scarcity of age 0+ Dolly Varden at individual sites and not territoriality. Aggression between these fish and fish of any other group was never observed. As a result of these complications, the DTNCF was not considered in further analyses.

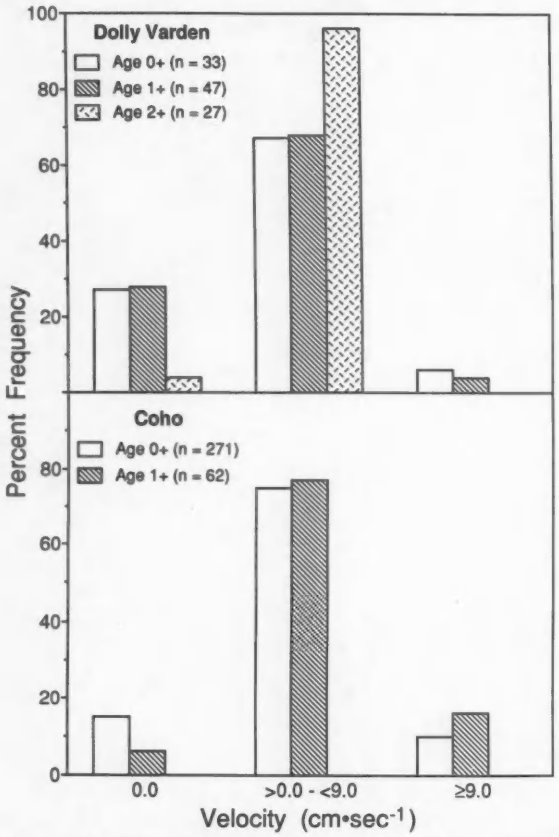

FIG. 4. Frequency distribution for water current velocity at the focal points coho salmon and Dolly Varden.

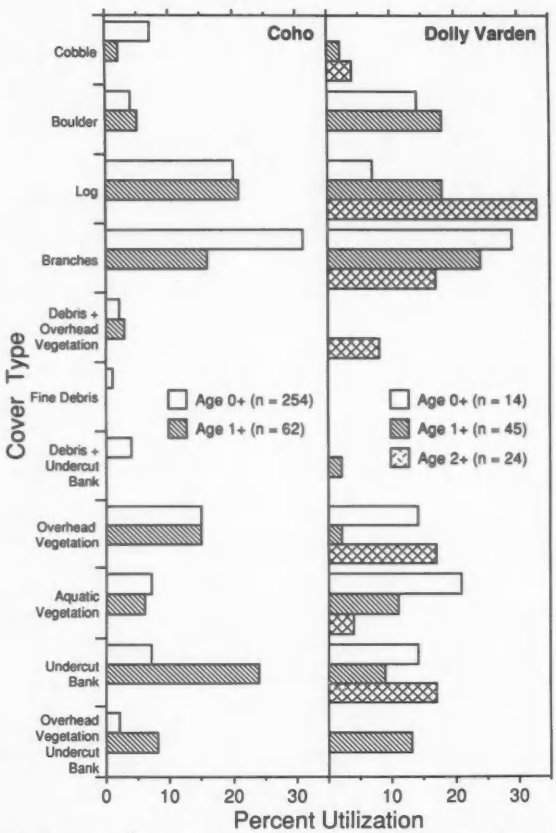

FIG. 5. Percent usage of 11 cover types by coho salmon and Dolly Varden.

Water Velocity

Over 85% of the coho salmon and 95% of the Dolly Varden occupied focal points where the water velocity was between 0.0 and 9.0 $cm \cdot s^{-1}$ (Fig. 4). Although maximum water velocity was not measured, focal points of the larger, more aggressive fish were located adjacent to areas of high water velocity at the upstream end of observation sites.

Cover

Logs, branches, undercut banks, dense overhead vegetation, and large boulders undercut by stream flow were the primary types of cover closest to fish of both species and of all age-classes (Fig. 5). In these "concealment" cover types the fish could completely hide themselves, at least from a streambank vantage point.

Logs and branches alone comprised over 50% of the cover utilized by age 0+ coho salmon (Fig. 5). Older coho salmon also used logs and branches, which together with undercut banks accounted for over 60% of the cover used by yearling coho salmon. The use of concealment cover by yearling coho salmon was even greater than that indicated by the percent utilization data. When yearling coho salmon were disturbed, for example, by sudden movements of the observer on the streambank, they usually darted to cover. In several instances these yearling coho salmon by-passed the nearest cover and, instead, used more distant concealment cover.

Branches and aquatic vegetation comprised 49% of the cover used by age 0+ Dolly Varden. In contrast, over 70% of the age 1+ and 85% of the age 2+ Dolly Varden used concealment cover.

Substrate

Sixty-five percent of the age 0+ and 55% of the age 1+ coho salmon were found over small and medium gravel (Fig. 6). Similarly, 61% of the age 1+ and 55% of the age 2+ Dolly Varden used small and medium gravel. Age 0+ Dolly Varden were found over small gravel which together with rock and fine debris comprised their total substrate use.

Discriminant Analysis

Stepwise discriminant analysis on microhabitat variables for the two age-groups of coho salmon and three age-groups of Dolly Varden demonstrated that all seven variables contributed significantly to group separation (Table 4). Although differences existed among all five groups, the principal separation occurred between species. Age-classes within a species merely occupied different locations on what was essentially a species continuum.

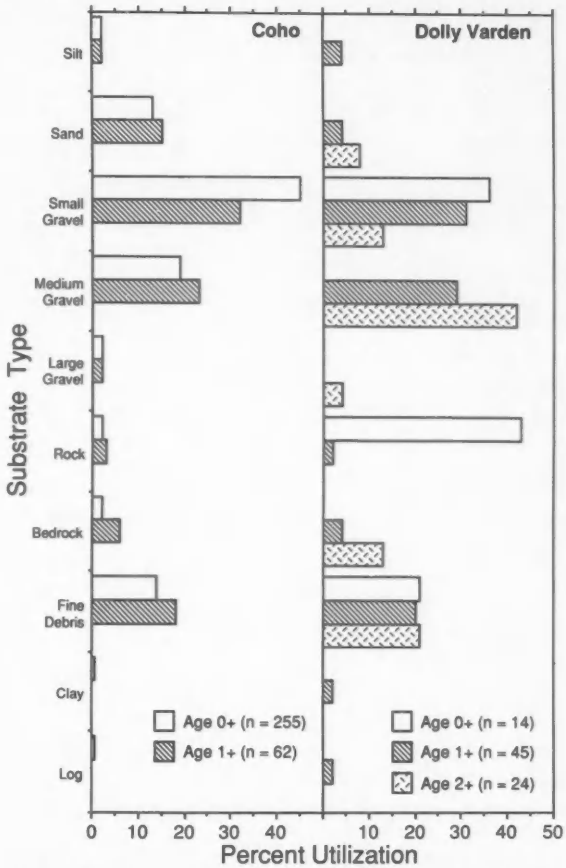

FIG. 6. Percent usage of 10 substrate types by coho salmon and Dolly Varden.

TABLE 4. Standardized discriminant function coefficients and percent of variation explained. Grouping and sample size (*N*) were coho salmon age 0+ (246), coho salmon age 1+ (60), Dolly Varden age 0+ (13), Dolly Varden age 1+ (45), and Dolly Varden age 2+ (25).

Variable	Discriminant function I	Discriminant function II
Focal point depth (FPD)	−2.088	−0.426
Depth (D)	1.675	1.293
Distance to nearest cover (DTNC)	0.344	−0.060
Distance to nearest fish (DTNF)	0.136	0.581
Substrate (S)	−0.120	−0.128
Velocity (V)	0.036	0.078
Cover (C)	0.030	0.084
Percent of variance	88.16%	8.14%

The absolute values of the standardized discriminant function coefficients show that, in order of decreasing importance, FPD, D, DTNC, and DTNF contributed the most to group separation in the first discriminant function, which primarily demonstrated differences between species (Fig. 7A). Compared to Dolly Varden, coho salmon of both age-classes were: higher in the water column, in shallower water, further from the nearest cover, and further from other fish.

Within-species differences along the first discriminant function also were apparent but less clearly defined. The age-classes of each species were discriminated from each other with regard to the variable gradients except for the DTNF for coho salmon. Age 1+ coho salmon actually were further from other fish than were age 0+ coho salmon. This anomaly occurred because the DTNF was the fourth most important variable in the first function and therefore had less influence relative to the first three variables in distinguishing group differences.

Depth, DTNF, and FPD were the most important variables in the second discriminant function, which more accurately described within-species differences (Table 4). The older fish of both species were in deeper water, further from other fish, and at deeper focal point depths (Fig. 7B). However, the function did not make a clear distinction between species, as evidenced by the position of the centroid for subyearling coho salmon relative to those for age 1+ and 2+ Dolly Varden. Depth was the most important variable in this function but the distance to nearest fish was probably responsible for the more positive position of yearling coho salmon along the discriminant function axis in relation to all other groups. Centroids on the second axis were necessarily closer together than on the first, as the second function contained only 8% of the total discriminatory power (Table 4).

The percent classification by group demonstrates the major separation by species with less distinction between individual age-classes (Table 5). Subyearling coho salmon were most frequently misclassified as yearling coho salmon and vice-versa. Over 85% of all coho salmon cases were classified as coho salmon. Likewise, Dolly Varden misclassification mostly fell into other Dolly Varden groups. No age 2+ Dolly Varden were misclassified as coho salmon and only 11.1% of age 1+ Dolly Varden were misclassified as subyearling and yearling, coho salmon.

Laboratory

The FPD of age 0+ coho salmon varied between trials. In Trial 1, the FPD of age 0+ coho salmon when alone did not differ ($P>0.05$) from when Dolly Varden were present. In the second trial, age 0+ coho salmon were higher in the water column when the two species were together compared to when coho salmon were alone ($P<0.05$).

Dolly Varden held positions closer to the bottom of the artificial stream channels and were more closely associated with cover than were coho salmon (Table 6). Although choice of position in the artificial channels by one species was not affected by the presence of the other species, the FPDs of Dolly Varden were lower when coho salmon were present (Table 6); only in Trial 2, however, was the difference significant ($P<0.05$). As in the field, Dolly Varden foraged primarily on the bottom, making an occasional foray into the water column to inspect items in the drift. Each species was further from cover when together than when alone, but the differences were not significant.

Behavioral Interactions

Coho Salmon

Chases, nips, and frontal displays (Hartman 1965) were the primary behaviors exhibited by coho salmon. When the species were together, the fraction of interactions directed toward Dolly Varden was relatively small, 7.5% and 13.4% in Trials 1 and

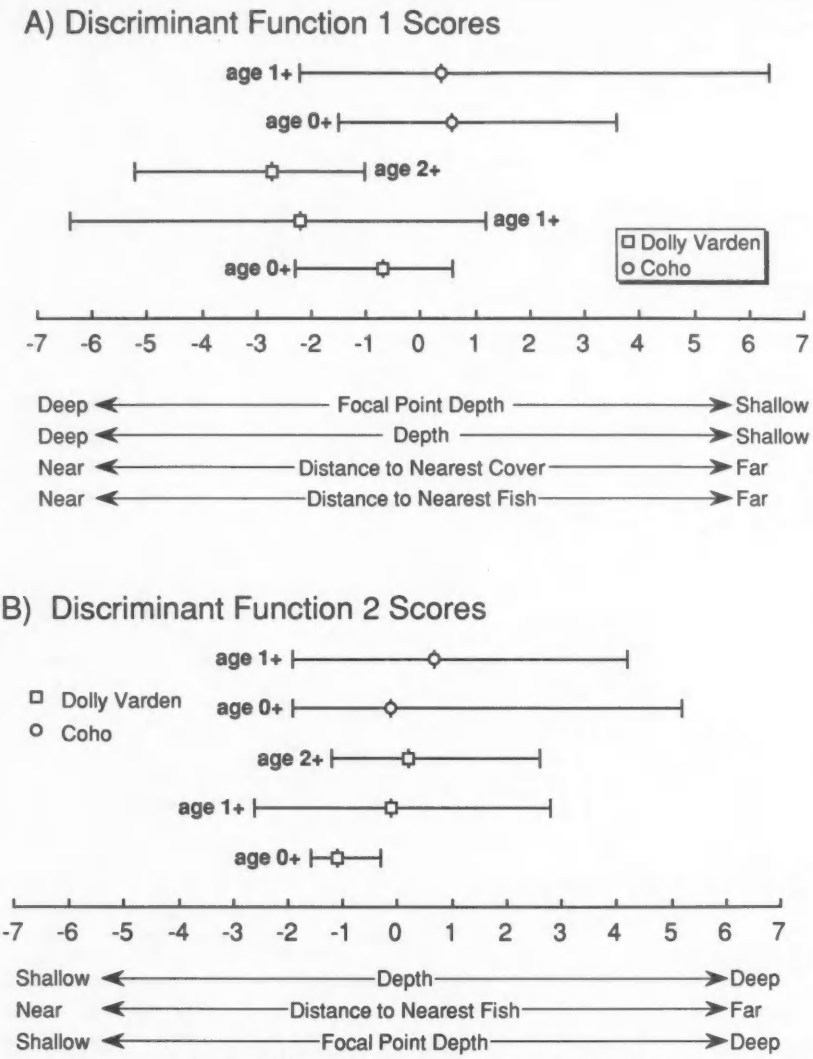
A) Discriminant Function 1 Scores
age 1+
age 0+
age 2+
age 1+
age 0+
Dolly Varden
Coho
-7 -6 -5 -4 -3 -2 -1 0 1 2 3 4 5 6 7
Deep Focal Point Depth Shallow
Deep Depth Shallow
Near Distance to Nearest Cover Far
Near Distance to Nearest Fish Far
B) Discriminant Function 2 Scores
age 1+
Dolly Varden
Coho
age 0+
age 2+
age 1+
age 0+
-7 -6 -5 -4 -3 -2 -1 0 1 2 3 4 5 6 7
Shallow Depth Deep
Near Distance to Nearest Fish Far
Shallow Focal Point Depth Deep

FIG. 7. Means (centroids) and ranges of positions on (A) the first, and (B), the second discriminant function axis for five groups of coho salmon and Dolly Varden.

2, respectively. The presence of Dolly Varden did not influence the mean number of behavioral interactions initiated by coho salmon ($P>0.05$) (Table 7).

Dolly Varden

Nips and lateral displays were the most common types of behaviors exhibited by Dolly Varden in interspecific encounters. The mean number of behavioral interactions per Dolly Varden was significantly greater (t-test; $P<0.05$) in Trial 1 when coho salmon were present than when Dolly Varden were alone (Table 7). In Trial 2, however, there was no difference ($P>0.05$) when coho salmon were present and when they were absent. The difference in the mean number of interactions in Trial 1 was probably a statistical artifact rather than an indicator of the degree of behavioral interaction between the species. No behavioral interactions were observed when Dolly Varden were alone, and only four interactions were observed when coho salmon were present. Of these four interactions, two were directed towards coho salmon. In Trial 2, 11 of the 16 interactions observed when the species were together were directed towards coho salmon. A total of eight interactions were observed when Dolly Varden were alone.

Discussion

Although juveniles of coho salmon and Dolly Varden displayed overlap in several habitat parameters, observations of microhabitat distributions and foraging patterns indicated that each species primarily exploited resources not readily available to or selected by the other. In laboratory tests, each species occupied the same microhabitat whether alone or in the presence of the other species, suggesting that microhabitat selection

TABLE 5. Proportion of group membership predicted by discriminant function analysis for five groups of juvenile salmonids. Classification number in parentheses.

Actual group	Sample size *N*	Predicted group membership				
		CO 0+	CO 1+	DV 0+	DV 1+	DV 2+
Coho salmon age 0+ (CO 0+)	246	62.2% (153)	22.8% (56)	13.8% (34)	1.2% (3)	0.0% (0)
Coho salmon age 1+ (CO 1+)	60	31.7% (19)	55.0% (33)	8.3% (5)	3.3% (2)	1.7% (1)
Dolly Varden age 0+ (DV 0+)	13	7.7% (1)	0.0% (0)	69.2% (9)	23.1% (3)	0.0% (0)
Dolly Varden age 1+ (DV 1+)	45	6.7% (3)	4.4% (2)	22.2% (10)	37.8% (17)	28.9% (13)
Dolly Varden age 2+ (DV 2+)	24	0.0% (0)	0.0% (0)	20.8% (5)	20.8% (5)	58.3% (14)

TABLE 6. *T*-test for depth of focal point and distance to nearest cover for juvenile coho salmon and Dolly Varden when alone and together in laboratory streams.

		Depth of focal point			Distance to nearest cover		
		Alone	Together	*P*	Alone	Together	*P*
		Age 0+ Coho salmon					
Trial 1	Mean	5.5	4.6	>0.05	12.9	15.3	>0.05
	SD	3.3	2.5		11.5	11.7	
	N	45	53		45	53	
Trial 2	Mean	5.4	7.3	<0.05	15.2	20.0	>0.05
	SD	3.1	4.5		13.0	15.9	
	N	40	51		40	51	
		Age 1+ Coho salmon					
Trial 1	Mean	6.9	4.6	>0.05	14.7	18.4	>0.05
	SD	3.5	2.5		10.5	12.1	
	N	45	25		45	25	
Trial 2	Mean	5.0	6.9	>0.05	15.8	22.0	>0.05
	SD	3.5	4.2		15.0	16.0	
	N	26	31		26	31	
		Dolly Varden					
Trial 1	Mean	1.0	0.4	>0.05	4.4	5.3	>0.05
	SD	2.0	2.2		8.7	10.0	
	N	52	58		52	58	
Trial 2	Mean	2.8	1.4	<0.01	8.9	9.5	>0.05
	SD	3.5	1.8		10.2	10.1	
	N	59	58		59	58	

TABLE 7. *T*-test for mean number of interactions per fish per observation period (3 min) by juvenile Dolly Varden and coho salmon when alone and together in laboratory streams. Standard deviations are in parentheses.

	Coho salmon			Dolly Varden		
	Alone	Together	*P*	Alone	Together	*P*
Trial 1	0.47 (0.36)	0.90 (0.70)	>0.05	0.0 (0.0)	0.39 (0.45)	<0.05
Trial 2	0.80 (0.61)	1.28 (0.63)	>0.05	0.19 (0.49)	0.54 (0.66)	>0.05

was innately controlled as a consequence of a long evolutionary history of coexistence.

The microhabitats of juvenile coho salmon and Dolly Varden were distinguished by four principal resource dimensions: depth of focal point; water depth; distance to nearest fish; and distance to nearest cover. Compared to Dolly Varden, coho salmon were higher in the water column, in shallower water, and were further from cover and other fish. These differences in microhabitat use reflected the foraging behavior of the two species in small streams.

Coho salmon actively maintained and defended positions in the water column which facilitated their exploitation of drift organisms. Large coho salmon tended to occupy positions closer to cover than their smaller conspecifics, but the dependence upon cover apparently was tempered by the need to be near areas where drift was concentrated, such as the heads of pools. Dolly Varden, particularly the older (larger) individuals, tended to be more secretive than both similarly sized coho salmon and their smaller conspecifics, rarely straying far from protective cover. Dolly Varden foraged over the stream bottom, mouthing and then either swallowing or rejecting potential food items.

Within its particular microhabitat, each species presumably had an advantage in exploiting the available food resources. Field observations revealed, however, that both were somewhat opportunistic and captured food items from other locations in the streams. Coho salmon would occasionally leave their focal points to pick organisms from the stream bed. Similarly, Dolly Varden would dart to the surface to capture conspicuous items in the drift. Individuals of both species were particularly attracted to movement, and all fish would rush to the spot where any object hit the water surface. Attraction to movement is a commonly observed trait among salmonids (Chapman 1966).

The existence of inter- and intraspecific size groups also contributed to habitat partitioning. Large differences in lengths were noted for all groups except for the yearlings of both species. Considering all microhabitat variables simultaneously with discriminant analysis, the greatest group segregation occurred between the smallest coho salmon group (age 0+) and the largest Dolly Varden group (age 2+) (Table 6). None of the individuals in these groups was ever misclassified as belonging to the other group (Table 5). Everest and Chapman (1972) attributed segregation of juvenile chinook (*Oncorhynchus tshawytsha*) and steelhead in Idaho to size-related spatial requirements. These fish had similar habitat preferences at a

given size but avoided severe interactions by varying times of emergence and smoltification. Similar habitat segregation by size has been described for juvenile chinook and coho salmon (Lister and Genoe 1970) and single species populations of juvenile Atlantic salmon (*Salmo salar*) (Saunders and Gee 1963; Symons and Heland 1978; Wankowski and Thorpe 1979) and brook trout (*Salvelinus fontinalis*) (Saunders and Smith 1955).

Behavioral interactions among the coho salmon-Dolly Varden groups were infrequent and only a small fraction of the interactions observed were interspecific. This pattern would be expected if two species segregate selectively. Both in the field and in laboratory streams coho salmon and Dolly Varden used different portions of the water column, varied in their association with cover, and searched for food in different areas. Opportunities for interspecific interactions were therefore minimal. Everest and Chapman (1972) and Schutz and Northcote (1972) observed few interactions between salmonid species that segregate primarily by selective mechanisms. In contrast, behavioral interactions are common among species that interactively segregate (e.g. Baltz et al. 1982; Reeves et al. 1987).

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Periphyton Responses to Higher Trophic Levels and Light in a Shaded Stream[1]

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Hill, W. R., and B. C. Harvey. 1990. Periphyton responses to higher trophic levels and light in a shaded stream. Can. J. Fish. Aquat. Sci. 47: 2307–2314.

The effects of higher trophic levels on benthic primary producers were examined in the context of light limitation in a shaded headwater stream. Abundance (presence or absence) of a predatory fish *Semotilus atromaculatus* and a grazing snail *Elimia clavaeformis* were manipulated with in situ Plexiglas channels, while spatial variability in ambient light was related to variability in primary productivity among channels. Indirect effects of fish on periphyton were insignificant, possibly because grazers that are vulnerable to fish predation were scarce during the experiment, or because fish preyed upon other predators of grazers. Snails had compensatory effects within the periphyton: they diminished biomass and productivity in the loosely attached layer, but stimulated productivity in the tightly attached layer. Snails appeared to mitigate shading of the tightly attached layer by the loosely attached layer. Benthic primary productivity was highly correlated with ambient light levels, accounting for 42% of the variability in total (loosely and tightly attached layers combined) primary productivity. Multiple regression analysis indicated that fish and snail effects on total primary productivity were minor compared to light effects. These results suggest that light availability controls realized as well as potential production in this unproductive stream.

Les effets des niveaux trophiques supérieurs sur les producteurs primaires benthiques ont été étudiés dans le contexte d'un éclairage limité dans le cours supérieur ombragé d'un cours d'eau. L'abondance (présence ou absence) de poisson prédateur *Semotilus atromaculatus* et d'escargot brouteur *Elimia clavaeformis* a été manipulée avec des chenaux de Plexiglas in situ, tandis que la variabilité spatiale de la lumière ambiante était liée à la variabilité de la productivité primaire d'un chenal à l'autre. Les effets indirects du poisson sur le périphyton étaient peu importants, probablement parce que les brouteurs qui sont vulnérables à la prédation par les poissons étaient peu nombreux pendant l'expérience, ou parce que les poissons s'attaquaient à d'autres prédateurs des brouteurs. Les escargots avaient des effets compensateurs dans le périphyton : ils diminuaient la biomasse et la productivité dans la couche peu adhérente, mais stimulaient la productivité dans la couche très adhérente. Les escargots semblaient atténuer l'ombrage de la couche très adhérente par la couche peu adhérente. La productivité benthique primaire était hautement correlée au taux de lumière ambiante, représentant 42% de la variabilité (couches très et peu adhérentes combinées) de la productivité primaire totale. Une analyse de régression multiple a révélé que l'effet des poissons et des escargots sur la productivité primaire totale était peu important comparativement aux effets de la lumière. D'après ces résultats, il semble que la disponibilité de la lumière contrôle la production obtenue, aussi bien que la production potentielle, dans ce cours d'eau improductif.

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Relative importance of biotic and abiotic control of community function is unresolved in streams, as it is in many ecosystems. In the relatively well-studied pelagic zone of lakes, biotic factors can be pervasive at times, with effects of top-level carnivores "cascading" all the way down to primary producers (Carpenter et al. 1987). Abiotic factors (such as nutrient supply) in the limnetic pelagic zone appear to set ultimate limits on biomass and production, whereas biotic factors (such as predation and competition) determine specific trophic structure and rates within the limits set by abiotic factors (Carpenter et al. 1985; McQueen et al. 1986, 1989). Corresponding studies of "cascading" and other indirect effects of biotic interactions in stream ecosystems are rare (but see Power and Matthews 1983; Power et al. 1985; and Bowlby and Roff 1986), as are experimental studies that set biotic interactions in the context of abiotic constraints.

Surprisingly few studies have investigated light as a factor limiting lotic primary production. Nutrient limitation has received much more attention from stream ecologists (e.g. Elwood et al. 1981; Peterson et al. 1983; Pringle and Bowers 1984; Grimm and Fisher 1986), despite the integral role of riparian shade in the river continuum concept (Vannote et al. 1980). Shade from overhanging trees in headwater streams can be very extensive, reducing light reaching the streambed to less than 5% of full sunlight. Nutrient amendments in oligotrophic streams often do not increase algal biomass unless light is first augmented (Gregory 1980; Triska et al. 1983; Hill and Knight 1988b). In a multi-stream examination of the effect of grazing invertebrates, Feminella et al. (1989) found periphyton biomass to be negatively correlated to canopy cover, and were unable to demonstrate a significant effect of grazers in a highly shaded stream.

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In this experiment, we simultaneously manipulated the abundances of a common predator and a dominant herbivore in a shaded, headwater stream. The predators were juvenile creek chubs (*Semotilus atromaculatus*); creek chubs are widespread in North America (Lee et al. 1980), and can significantly reduce the densities of benthic invertebrates (Gilliam et al. 1989; Schlosser and Ebel 1989). The herbivore was *Elimia clavaeformis*, a prosobranch snail that scrapes microalgae from benthic substrates. *Elimia* spp. attain high densities in southeastern United States streams (Newbold et al. 1983; Richardson et al. 1988). *Elimia clavaeformis* is not consumed by creek chubs.

We looked for direct and indirect effects of fish and snail manipulations on benthic primary producers, and compared their effects to those of natural variation in light. If juvenile creek chubs consume significant numbers of predation-vulnerable grazing insects, then removing these fish should result in lower periphyton biomass or decreased snail growth. Periphyton biomass should increase in the absence of snails, but primary production may be enhanced (Lamberti et al. 1989). If higher trophic levels control periphyton dynamics, then the variance in primary productivity explained by the fish and snail manipulations should exceed the variance explained by light.

Study Area

Ish Creek is a second-order, low-gradient stream located in the Oak Ridge National Environmental Research Park (35°54′N, 84°22′W). It flows through a relatively undisturbed hardwood forest, and consists of shallow pools and riffles. The water in the stream is clear and warm (averaging 21°C during this study). Ish Creek drains a basin underlain by dolomite and limestone; alkalinity is moderately high (ca. 2.8 meq/L), and nutrient concentrations are moderately low (ca. 30 μg/L dissolved inorganic nitrogen and 5 μg/L soluble reactive phosphorus, Loar et al. 1990). Because of the extensive forest canopy, the stream receives less than 5% of full sunlight during the summer. Mean discharge during the study was low (2–3 L/s), but thunderstorms on several dates caused brief twentyfold increases in flow.

Methods

Flow-through Plexiglas channels served as experimental units in this experiment. The channels were constructed in pairs, with each side 122 cm long by 30 cm wide by 41 cm high. The area of the bottom of each channel was 0.37 m^2. Plastic mesh (6.4-mm openings) was clamped onto the ends of each channel to allow water and small organisms to move into and out of the channels. Channel pairs were placed in shallow pools (ca. 30 cm deep) so that the channel walls extended above the water surface. The long axis of the channels paralleled the direction of flow in Ish Creek. Wet gravel from the stream was poured into the channels to a depth of 4 cm, and a single layer of cobbles taken directly from the stream bed was placed on top of the gravel. Ten pairs of the channels were distributed longitudinally over a 500-m stretch of Ish Creek.

Fish and snails were applied to the channels in a split-plot factorial design. Each of the ten channel pairs functioned as a mainplot: two juvenile creek chubs (*Semotilus atromaculatus*) were added to both sides of five randomly chosen pairs, while no fish were added to the other five pairs. The sides of each channel pair functioned as subplots: 144 snails (*Elimia* sp.) were added to one side, while no snails were added to the other side. In summary, each channel contained one of four possible treatment combinations: (1) no fish or snails added, (2) fish alone added, (3) snails alone added, (4) both fish and snails added.

The two fish and 144 snails per channel approximated the natural densities of these animals in Ish Creek, as determined immediately before the experiment. Fish densities were estimated by electrofishing, and snail densities were estimated by counting the number of snails in 10 quadrats which were 0.05 m^2. Snails were collected and added to the channels on 15 June 1988. Thirty of the 144 snails added to each chamber were first marked with red acrylic fingernail paint and weighed as a group. Fish were collected by electroshocking and were added to the appropriate channel pairs on 21 June 1988.

We maintained original treatments during the experiment by removing snails and replacing fish as necessary. Snails small enough to pass through the mesh of the channels chosen to be snail-free were counted and removed every other day. Fish were checked periodically, but only one fish was lost during the experiment. It was replaced within a week of its loss. The Plexiglas sides of the channels were cleaned with a plastic scouring pad every week to prevent the accrual of periphyton that could function as an additional food supply for the snails.

Photosynthetically active radiation (PAR) was estimated at each channel location at the end of the experiment with ozalid paper light meters. Three meters were suspended over the center of each side of each channel pair for 24 h on 2 August, a typical sunny day. The ozalid paper light meters provided an integrated measurement of daily photon flux density (Friend 1961), and were calibrated with a LiCor quantum sensor.

Periphyton primary productivity and chlorophyll *a* were measured during the afternoon of 5 August, 7 wk after the treatments were initiated. In each side of each channel pair, two randomly chosen cobbles (ca. 5 cm diameter) were gently placed into a crystallizing dish already submerged in the channel. The dish was then removed from the water, and all but 500 mL of water was carefully withdrawn with a suction pump. The dish was then placed in shallow water adjacent to the channels and 185 kBq of $NaH^{14}CO_3$ was added. Alkaline pH (8.01–8.29) precluded loss of $^{14}CO_2$ from the incubation dishes by diffusion. Incubation times ranged from 2.75 to 3.5 h. At the end of the incubation period, loosely attached periphyton was dislodged from the cobbles in the dishes with several jets of water from a syringe. The cobbles were then removed from the dishes, rinsed in stream water, and placed in a jar with 30 mL of DMSO for the simultaneous extraction of incorporated ^{14}C and chlorophyll *a* (Palumbo et al. 1987). A 200-mL aliquot of the water in the incubation dish containing the loose periphyton was filtered through a glass-fiber filter (Whatman GFC). The filter was then placed in 10 mL of DMSO for ^{14}C and chlorophyll *a* extraction. Dark controls were run with rocks in three aluminum-covered crystallizing dishes, and were used to correct for residual, unincorporated ^{14}C and nonphotosynthetic uptake. Total inorganic carbon available for photosynthesis for each incubation was estimated from measurements of total alkalinity, pH, and temperature (Wetzel and Likens 1979). ^{14}C activity in the DMSO extract was assayed by liquid scintillation with external standardization. Phaeopigment-corrected chlorophyll *a* was determined spectrophotometrically (Strickland and Parsons 1972). Surface area of the cobbles was estimated by covering the top of each cobble with aluminum foil and then weighing the foil. Periphyton samples for gravimetric analysis were collected from the channels on 8 August. A single rock was randomly chosen from each channel, and the

TABLE 1. Number of snails in channels at end of the experiment.

Channel pair	Snail	No snail
1	141	2
2	116	2
3	129	4
4	220	25
5	122	3
6	104	1
7	136	5
8	113	0
9	110	0
10	98	0
$\bar{X}$ ± SE	129 ± 11	4.2 ± 2.4

loosely attached periphyton was dislodged and then collected by filtration. Strongly attached periphyton was removed from the rock with a stout brush and also collected by filtration. Filters (Whatman GFC) were weighed before filtration and after the filtered periphyton dried at 60°C for 24 h.

Snails were collected from the channels on 8 August, blotted dry, and weighed (blotted wet mass). Marked and unmarked snails were weighed in separate groups. Initial and final weights of the snails were converted to ash-free dry mass (AFDM) with a wet mass to ADFM regression previously computed for Ish Creek *Elimia* (W. R. Hill, unpubl. data). Mean growth per snail was calculated only for marked snails.

Results

Treatments

Fish and snail treatments remained consistent throughout the experiment. Except for the one fish that was replaced, all fish originally added to the channels were recovered, and no immigrants were found. Although small snails entered the channels, fewer than 10 were generally found in (and removed from) any channel during any 2-d period. Emigration and mortality of snails in the channels appeared low, since 70% of the marked snails were recovered at the end of the experiment. There was no evidence of snail reproduction during the experiment. As a result of minimal immigration, emigration, reproduction, and mortality, substantial differences in snail densities between the two sides of each channel pair were maintained to the end of the experiment (Table 1).

Independence of Fish and Snails

There were no significant interaction terms in the analysis of variance (ANOVA) of fish and snail effects on periphyton parameters (Table 2, last column). Because of the absence of significant interactions, we evaluated fish and snail effects independently.

Absence of Significant Fish Effects

The presence of fish did not produce obvious cascading effects: the effect of fish on periphyton biomass and productivity parameters was not statistically significant (Table 2, first column).

Snail Effects

Snail manipulations produced visible effects on periphyton: cobbles in channels lacking snails had obvious accumulations of loosely attached silt, fine detritus, and diatoms. The AFDM of this loosely attached layer of periphyton was approximately 80% greater in channels without snails than in channels with snails (Fig. 1). Although the mean AFDM of the tightly attached periphyton was somewhat greater in channels without snails than in channels with them (Fig. 1), the difference between the means was not significant (Table 2). Total periphyton AFDM (loosely and tightly attached layers combined) was dominated by the loosely attached layer (80% of the total), and was therefore also two times greater in channels without snails (Fig. 1).

Snails prevented the accumulation of chlorophyll *a* in the loosely attached layer, but had little effect on chlorophyll *a* in a tightly attached layer (Fig. 1, Table 2). In contrast to periphyton AFDM, most (95%) of the chlorophyll *a* was in the tightly attached layer. The effect of snails on total periphyton chlorophyll *a* was therefore insignificant (Table 2). DMSO extraction of the tightly attached layer may be more efficient than brushing (Palumbo et al. 1987), and could be partly responsible for differences in the proportions of chlorophyll *a* AFDM in the tightly attached layer.

Snails had reciprocal effects on areal-based primary productivity in the loosely attached and tightly attached layers of periphyton. They reduced productivity in the loosely attached layer by more than 80%, but increased productivity in the tightly attached layer by 40% (Fig. 2). Most (74%) of the total periphyton productivity was due to productivity in the attached layer. Therefore, the stimulatory effect of snails on the tightly attached layer, although weaker than the snails' depressing effect on the loosely attached layer, increased the contribution of the tightly attached layer to the extent that snail effects on total periphyton productivity were insignificant (Fig. 2, Table 2).

TABLE 2. Analyses of variance for the effects of fish, snails, and the fish × snail interaction on periphyton. All analyses were performed on logarithmically transformd data. AFDM = ash-free dry mass.

	Fish		Snails		Fish × snails	
	F	(*P*)	F	(*P*)	F	(*P*)
AFDM ($mg \cdot cm^{-2}$)						
Loosely attached	0.52	(0.49)	13.86	(0.006)	3.02	(0.12)
Tightly attached	0.01	(0.93)	3.54	(0.10)	0.03	(0.88)
Total	0.29	(0.61)	11.53	(0.009)	2.02	(0.19)
Chlorophyll *a* ($\mu g \cdot cm^{-2}$)						
Loosely attached	0.00	(0.99)	48.14	(0.0001)	0.26	(0.63)
Tightly attached	0.27	(0.62)	0.48	(0.51)	0.27	(0.62)
Total	0.28	(0.61)	1.34	(0.28)	0.00	(0.99)
Primary productivity ($\mu g\ C \cdot cm^{-2} \cdot h^{-1}$)						
Loosely attached	0.56	(0.48)	31.73	(0.0005)	0.28	(0.61)
Tightly attached	3.31	(0.11)	4.97	(0.056)	0.00	(0.99)
Total	2.82	(0.13)	0.11	(0.75)	0.00	(0.99)
Production·chl^{-1} ($\mu g \cdot \mu g^{-1} \cdot h^{-1}$)						
Loosely attached	1.74	(0.22)	10.99	(0.011)	0.00	(0.99)
Tightly attached	3.71	(0.09)	59.83	(0.0001)	0.01	(0.92)
Total	3.67	(0.09)	1.46	(0.26)	0.00	(0.99)

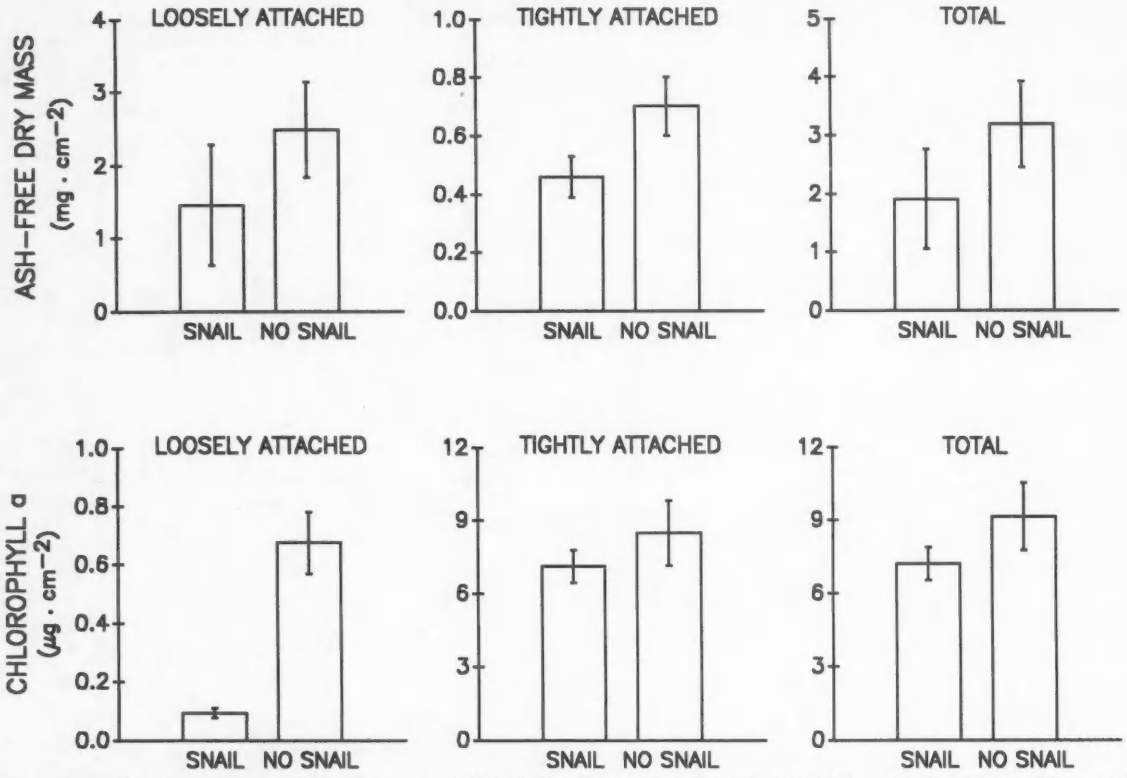

FIG. 1. Snail effects on periphyton ash-free dry mass and chlorophyll *a*. Total = loosely attached + tightly attached periphyton. Means ± 1 SE are shown.

Snails increased chlorophyll-specific productivity in both layers by over 60% (Fig. 2). Despite the significantly increased chlorophyll-specific productivity of both layers analyzed individually, their combined (total) chlorophyll-specific productivity was not significantly greater when snails were present (Fig. 2, Table 2).

Light Effects

Although light was low at all channel locations, variation between locations was relatively large: integrated PAR ranged from 0.7 to 3.4 $E \cdot m^{-2} \cdot d^{-1}$. We used this channel-to-channel variability to explore the importance of light to Ish Creek periphyton: rank correlations of integrated PAR with biomass and productivity parameters were performed. Light was not significantly correlated with chlorophyll *a* in a loosely attached layer, but a significantly positive relationship existed between light and chlorophyll *a* in the tightly attached layer (Fig. 3). Because the great majority of chlorophyll *a* was located in the tightly attached layer, total chlorophyll *a* was also significantly correlated with light. Light was not significantly correlated with AFDM in either layer of periphyton in the channels (Spearman's rho < 0.2, $P > 0.4$).

Primary productivity by periphyton in the loosely attached layer was not significantly associated with light, but light and productivity in the tightly attached layer were very highly correlated, as were light and total productivity (Fig. 3).

Regression Analysis

Total primary productivity (area-specific) in the channels was regressed against fish mass, snail mass, and light so that the response of periphyton to biotic manipulations could be evaluated in the context of an abiotic constraint. Light generated the only statistically significant partial regression coefficient in the multiple regression, accounting for 42% of the channel-to-channel variation in productivity (Table 3). Fish and snail manipulations together accounted for only 8% of the variation in total productivity. The three independent variables used in the multiple regression were not significantly correlated.

Primary productivity in the tightly attached layer was regressed against dry mass in the loosely attached layer and integrated PAR to determine if upper layers inhibited productivity by the tightly attached layer. Photosynthetically active radiation was again a significant variable in the regression, accounting for 44% of the channel-to-channel variation in tightly attached productivity (Table 3). Loose particulate mass was also a significant variable, and its negative coefficient indicated inhibition of productivity in the attached layer by the loosely attached layer. Loose particulate mass was responsible for 13% of variation in the regression. As in the first multiple regression, the independent variables were uncorrelated.

Snail Growth

Approximately 70% of marked snails were recovered at the end of the experiment. The mean growth of marked snails in

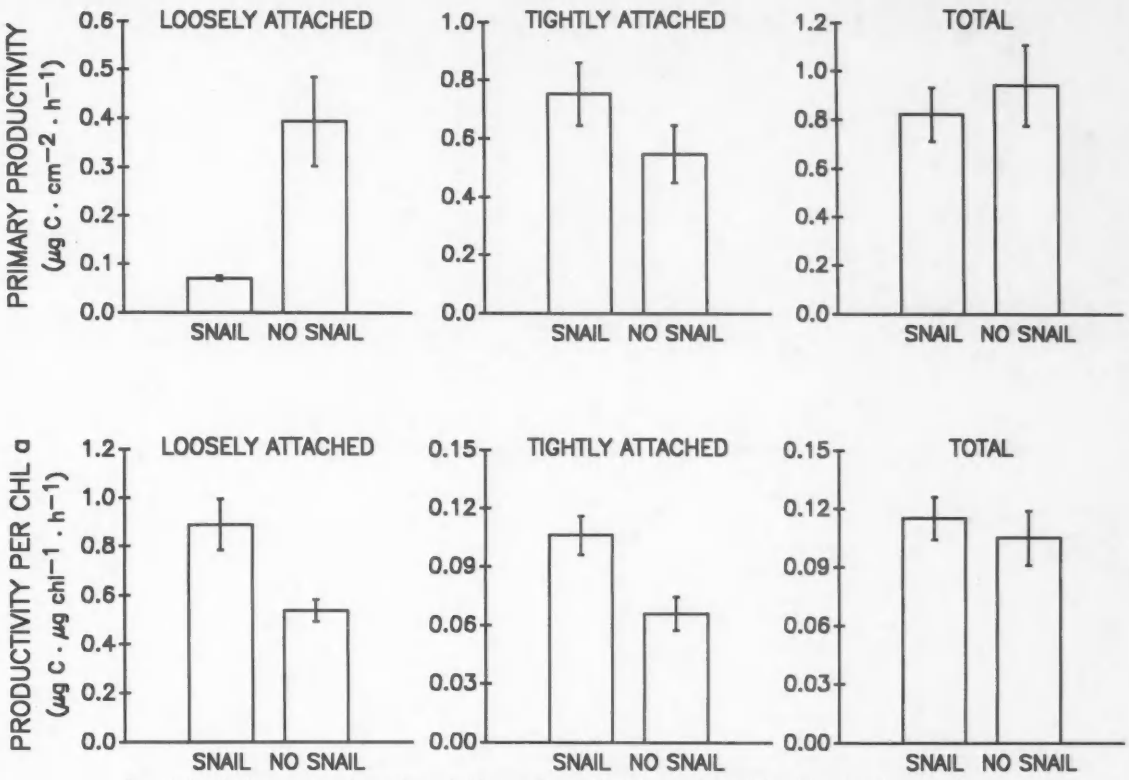

FIG. 2. Snail effects on areal-specific and chlorophyll-specific primary productivity. Means ± 1 SE are shown.

channels without fish was greater than the mean growth of marked snails in channels with fish (0.986 ± 0.135 mg AFDM/snail vs. 0.762 ± 0.086 mg AFDM/snail, mean ± standard error), but the difference was not statistically significant ($F = 1.95$, $P = 0.20$). Growth was only weakly correlated with light (Spearman's rho = 0.47, $P = 0.17$). The correlation between snail growth and total periphyton productivity was stronger (Spearman's rho = 0.563, $P = 0.09$), but still was not statistically significant.

Discussion

According to a simple model of cascading trophic effects, predation by creek chubs should have reduced the densities of grazing invertebrates, thereby indirectly increasing the biomass and productivity of epilithic algae. Gilliam et al. (1989) and Schlosser and Ebel (1989) found that juvenile creek chubs were capable of reducing the densities of benthic invertebrates in New York and Minnesota streams, and in this study significantly reduced the total biomass of benthic invertebrates (B. Harvey and W. Hill, unpubl. data). Despite the reduction in the biomass of benthic invertebrates, the channels lacking fish did not develop substantially larger numbers of grazing insects, even when snails were also absent.

There are several potential explanations for the lack of a fish effect on grazing invertebrates. Grazing insects in general, and taxa particularly vulnerable to fish predation (e.g. baetid and siphlonurid mayflies), were scarce in Ish Creek during this experiment. Thus, the pool of potential colonists may have been too small to respond to the fish manipulations. Alternatively, juvenile creek chubs may have occupied a somewhat higher trophic position than that shown in a simple trophic model, and any direct predation on invertebrate grazers by creek chubs may have been compensated by the positive indirect effect of their predation on other predators. Gomphid dragonfly larvae and young-of-the-year creek chubs, for example, were less abundant in channels containing juvenile creek chubs (B. Harvey and W. Hill, unpubl. data). It is also possible that juvenile creek chubs in Ish Creek during the summer may specialize in feeding on terrestrial invertebrates that fall into the stream, and may not prey heavily upon grazing invertebrates. In any case, a strong link between fish and periphyton was not detected in this experiment: we saw no evidence of indirect stimulation of primary productivity by secondary consumers.

The major direct effect of grazing by snails seemed to be that of preventing the accumulation of particulate matter in the loosely attached layer of periphyton. Snail effects on total periphyton (loosely and tightly attached layers combined) were significant only for AFDM, a parameter that was dominated by the loosely attached layer. Total chlorophyll and total productivity, parameters dominated by the tightly attached layer, were not significantly affected by snails. Other studies have also found disproportionately large effects of grazing on loosely attached or upper layers of periphyton by snails (Sumner and McIntire 1982; Steinman et al. 1987; Lowe and Hunter 1988) and certain mayflies (Coletti et al. 1987; Hill and Knight 1988a).

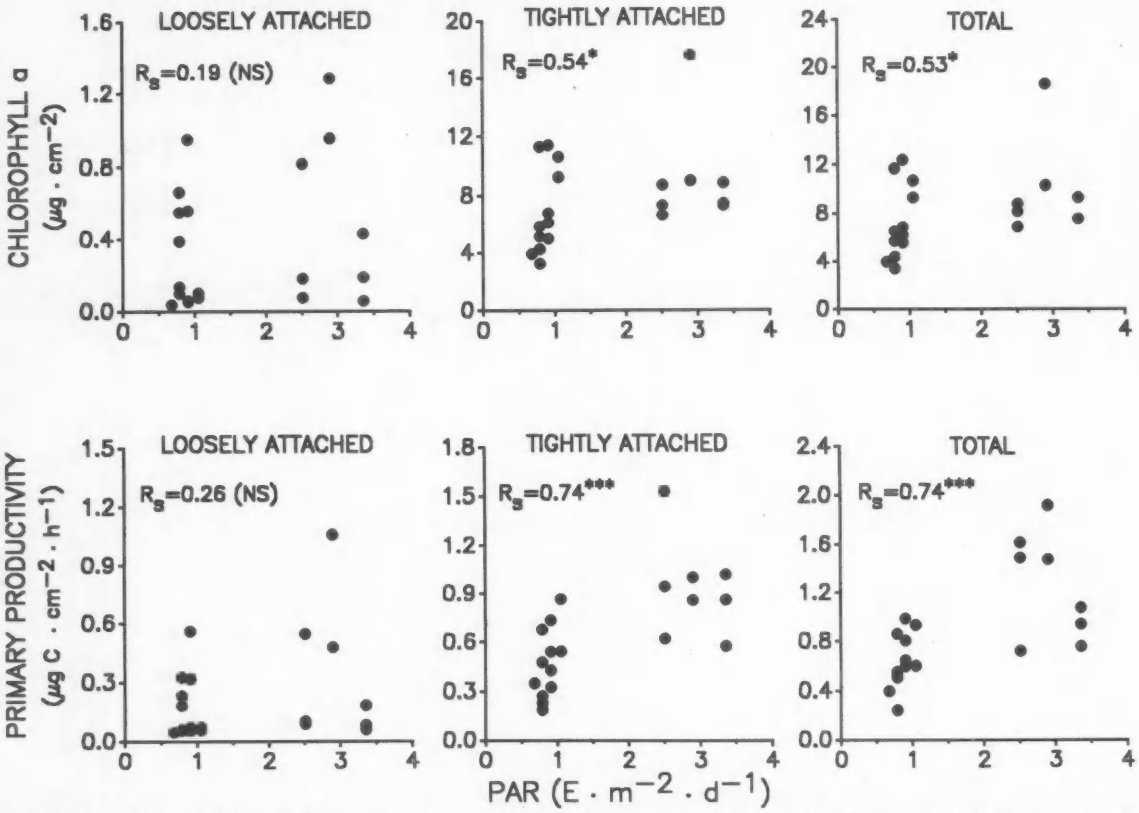

FIG. 3. Chlorophyll *a* and primary productivity of periphyton vs. photosynthetically active radiation (PAR) integrated over 24 h R_s = Spearman's rho. * = $P < 0.05$, *** = $P < 0.001$.

TABLE 3. Multiple regressions of benthic primary production ($\mu g\ C \cdot cm^{-2} \cdot h^{-1}$) vs. biotic and abiotic independent variables. PAR = photosynthetically active radiation. AFDM = ash-free dry mass. * = $P<0.05$, *** = $P<0.001$.

Regression 1: Ln total primary production = B_0 + $B_1 \times$ PAR + $B_2 \times$ fish + $B_3 \times$ snails

Independent variable	Regression coefficient	Partial r^2
PAR ($E \cdot m^{-2} \cdot d^{-1}$)	B_1 = 0.260*	0.42
Fish (g wetmass)	B_2 = −0.155	0.06
Snails (g AFDM)	B_3 = −0.202	0.02

Regression 2: Ln tightly attached primary production = B_0 + $B_1 \times$ PAR + $B_2 \times$ loosely attached dry mass

Independent variable	Regression coefficient	Partial r^2
PAR ($E \cdot m^{-2} \cdot d^{-1}$)	B_1 = 0.402***	0.44
Loosely attached dry mass ($mg \cdot cm^{-2}$)	B_2 = −0.014*	0.13

Biomass in the tightly attached layer of periphyton did not increase significantly in the absence of snails, suggesting that indirect, compensatory effects of grazing may have been important. In the absence of snails, chlorophyll-specific primary productivity in the tightly attached layer was only 60% of that when snails were present. Grazing by snails could have selected for algal taxa that were inherently more productive, but tightly attached algal assemblages in both grazed and ungrazed channels were dominated by the same taxa, the adnate diatom *Epithemia* and prostrate cells of *Stigeoclonium*. It seems more likely that the stimulated productivity in the tightly attached layer resulted from the snails increasing the supply rate of a productivity-limiting resource.

Gastropods can recycle limiting nutrients, either through diffuse excretion into the surrounding water (Grimm 1988), or through the deposition of nutrient-rich mucus (cf. Connor and Quinn 1984). However, the strong correlation between primary productivity and ambient light in this experiment suggests that light, rather than nutrients, limited primary productivity in the channels. Accordingly, the most likely means by which snails coud have stimulated primary productivity in the tightly attached layer is by increasing the supply rate of light to this layer. By preventing the accumulation of silt, fine detritus, and diatoms in the loosely attached layer, snails mitigated the shading effects produced by these particles. Evidence of the inhibitory effect of accumulated particulate matter in the loosely attached layer is provided by the significant negative regression coefficient for loosely attached dry mass in the regression on tightly attached productivity (Table 3).

Enhancement of primary production by grazing is an appealing idea with examples from terrestrial (McNaughton 1976) and aquatic (Porter 1977) habitats. Zooplankton in lakes can stim-

ulate phytoplankton productivity by recycling limiting nutrients (Lehman 1980; Carpenter et al. 1985), and Sterner (1986) showed that nutrient regeneration by *Daphnia* in an experimental microcosm nearly counterbalanced losses due to ingestion. Although increases in biomass-specific productivity due to grazing are commonly observed, actual increases in net production are rare in benthic studies (but see Flint and Goldman 1975; Lamberti et al. 1989). In this study, the increase in biomass-specific productivity due to the snails resulted in an increase in areal-specific productivity that was marginally significant ($P = 0.056$). Stimulation of biomass-specific productivity of the tightly attached layer in channels that contained snails appeared to compensate for the cropping of loosely attached algae by snails, causing the overall effect of snails on total areal-specific productivity to be insignificant.

Although adequate light is obviously necessary to sustain autotrophic communities, studies providing experimental or correlative evidence of light-limitation are scarce in stream ecology (but see Triska et al. 1983; Hill and Knight 1988b; Feminella et al. 1989). Nutrients have garnered the attention of most researchers looking for abiotic controls of lotic primary production (e.g. Elwood et al. 1981; Peterson et al. 1983; Pringle and Bowers 1984; Grimm and Fisher 1986). Ish Creek, like many undisturbed headwater streams, has relatively low concentrations of dissolved nitrogen and phosphorus. However, during spring and summer when the riparian canopy is fully developed, light is likely to be the principal abiotic factor limiting benthic primary productivity in Ish Creek. Photosynthetically active radiation in the section of Ish Creek used in this study was generally less than 50 $\mu E \cdot m^{-2} \cdot s^{-1}$, and photosynthesis by periphyton in shaded streams in the Oak Ridge vicinity saturates above 100 $\mu E \cdot m^{-2} \cdot s^{-1}$ (W. Hill, unpubl. data). Compared to the fish and snail manipulations, light accounted for the majority of the channel-to-channel variation in benthic primary productivity. This finding contrasts with the cascading trophic effects model for lakes in which abiotic factors control potential production, while biotic factors control realized production (Carpenter et al. 1985).

Despite the strong relationship between light and productivity, light and snail growth were only weakly related. Snails clearly depleted at least a portion of their periphyton food resource (the loosely attached layer) and exhibited food-limited growth at similar densities in a nearby stream (W. Hill unpubl. data). The lack of correspondence between light and snail growth was therefore somewhat surprising. Snails may gain most of their nourishment from the loosely attached layer of periphyton, however, and this layer did not appear light-limited. Also, snails grew little (only 10% increase in blotted wet mass) over the 7-wk period. The effect of light on small changes in snail mass may have been obscured by the variation inherent in measuring blotted wet mass and by the potential variation introduced by incomplete recovery of marked snails (ca. 30% were not recovered).

The response of periphyton to grazers and light was distinctly layer-specific. Chlorophyll and productivity in the loosely attached layer was controlled primarily by snail grazing. Neither chlorophyll nor productivity in this part of the periphyton was significantly related to light, suggesting that light was sufficient. In the tightly attached layer, however, chlorophyll and productivity were significantly related to ambient light. The *Epithemia* and *Stigeoclonium* cells that dominated biomass in this layer did not appear to be very susceptible to grazing by the snails, for reasonable amounts of chlorophyll persisted under relatively large standing stocks of snails. Algal cells in the tightly attached layer were inhibited by the accumulation of silt, detritus, and other microalgae in the absence of snails, presumably because of the shading caused by these particles. Because light was not significantly related to biomass and productivity in the loosely attached layer, a combination of terrestrial shading and self-shading appeared to cause light-limitation in the tightly attached layer.

In conclusion, food web manipulations in this experiment produced relatively mild responses in primary producers. Fish lacked a strong, direct link to primary producers, but low ambient light may also have muted the response of periphyton to positive, indirect predation effects. The loosely attached layer of periphyton was most affected by a biotic factor (snail grazing), whereas abiotic regulation (light) appeared most important in the tightly attached layer, the layer that dominated total algal biomass and productivity. The results from this experiment suggest abiotic control of both potential and actual primary production in Ish Creek.

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Migratory Catch-Age Analysis

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We review techniques for estimating the abundance of migratory populations and develop a new technique based on catch-age data from geographic regions and our earlier technique, catch-age analysis with auxiliary information (Deriso et al. 1985, 1989). Data requirements are catch-age data over several years, some auxiliary information, and migration rates among regions. The model, containing parameters for year-class abundance, age selectivity, full-recruitment fishing mortality, and catchability, is fitted to data with a nonlinear least squares algorithm. We present a measurement error model and a process error model and favor the process error model because all model parameters can be jointly estimated. By application to data on Pacific halibut, the process error model converges readily and produces estimates with no significant bias. These estimates have relatively high precision compared to those from analyses which did not incorporate migration information. The error structure used in a model has a more significant impact on parameter estimates than migration rates. A sensitivity study of migration rates shows sensitivity of the order of the rates themselves.

On passe en revue les techniques d'évaluation de l'abondance des populations migratoires et on élabore une nouvelle technique fondée sur les données relatives à la prise par âge provenant des diverses régions géographiques et sur notre technique antérieure, c'est-à-dire l'analyse des prises par âge au moyen de renseignements auxiliaires (Deriso et al. 1985, 1989). Les données nécessaires sont les données sur les prises par âge portant sur plusieurs années, certaines informations auxiliaires et les taux de migration entre les régions. Le modèle, qui comprend des paramètres relatifs à l'abondance de la classe d'âge, à la sélectivité en fonction de l'âge, à la mortalité par pêche en régime de plein recrutement et à la capturabilité est ajusté aux données à l'aide d'un algorithme non linéaire des moindres carrés. Nous présentons un modèle d'erreur de mesure et un modèle d'erreur de processus; nous préférons le modèle d'erreur de processus, car tous les paramètres de ce modèle peuvent être évalués conjointement. Lorsqu'il est appliqué à des données sur le flétan du Pacifique, le modèle d'erreur de processus converge facilement et produit des estimations sans erreur appréciable. Ces estimations ont une précision relativement élevée par rapport à celles des analyses qui ne comportaient pas de données sur la migration. La structure d'erreur employée dans un modèle a un impact plus appréciable sur les estimations des paramètres que les taux de migration. Une étude de la sensibilité des taux de migration montre une sensibilité de l'ordre des taux eux-mêmes.

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Incorporating the migration of a population into the estimation of its abundance should be critical for fisheries assessment. Surprisingly, the array of techniques available that use migration is fairly limited. One reason is that many populations are resident, especially in the adult stage. However, some notable exceptions include species of great commercial importance such as Pacific halibut (*Hippoglossus stenolepis*), Pacific salmon (*Oncorhynchus* sp.), tuna (*Thunnus* sp.), and sablefish (*Anoplopoma fimbria*). Another reason is that instantaneous assessments of a migratory population are often possible, especially when a population passes a location where it can be encountered. Finally, the mathematics of estimation for migratory populations can be complicated.

The purpose of this paper is to present a technique for estimating the abundance of a migratory population from catch-age data and migration rates. The technique is an extension of our earlier technique, catch-age analysis with auxiliary information (Deriso et al. 1985), which was developed for an entire population. The extension provides estimates of population parameters for geographic regions as well as the entire population.

Analysis of catch-age data has become the primary assessment tool for fisheries occurring in temperate marine waters (International Council for the Exploration of the Sea (ICES) 1988; Megrey 1989). A variety of approaches is available, from the classical virtual population or cohort analysis of Pope (1972), which we contend is useful only for historical data, to more recent methods that integrate several sources of information. Common to these approaches is a model for catch-age data as a function of parameters for year-class strength, gear

selectivity, age selectivity, full-recruitment fishing mortality, and catchability (Deriso et al. 1985, 1989). Catch-age data are not sufficient for estimating population parameters, requiring the use of auxiliary information from catch-effort, surveys, or a spawner-recruit relationship (Deriso et al. 1985, 1989). Most methods require that fishing mortality or exploitation fraction is separable into independent age and year effects, which reduces the total number of parameters to be estimated. Parameter estimation follows from the specification of an objective function, such as least squares or maximum likelihood, which explicitly contains a weighting scheme for the amount of influence of the auxiliary information.

First, we provide a cursory review of abundance estimation techniques for migratory populations. We develop a measurement error model, which assumes measurement error in the catch data but no process error (stochastic variation) in the dynamics of the fish population. A process error model is then developed, which assumes no measurement error in the catch data but incorporates process error in the dynamics to allow for stochastic variation in meeting the separability assumption. Models incorporating both measurement error and process error are considerably more complicated, involving the use of Kalman filters to estimate parameters (Bard 1974), and are not considered in this paper. Finally, we apply the models to Pacific halibut data and show the sensitivity of migratory catch-age analysis to migration rates and the error structure assumption.

Review of Methods for Estimating Abundance of Migratory Populations

Some techniques for estimating abundance provide instantaneous estimates at a given time, such as catch-per-unit-effort (CPUE) methods, line transect methods, and hydroacoustic survey estimates, and can be found in Seber (1982). These techniques can be applied to migratory populations, bearing in mind the limitations of each method. The interesting question with these methods is how to combine estimates from individual days or regions; these problems are discussed by Mundy (1985) and Quinn et al. (1982) in part. Catch-per-unit-effort methods suffer notoriously due to changes in catchability. While standardization of effort is routinely undertaken (Gavaris 1980; Quinn 1987), variations in catchability and the number of factors that affect the catch process can still occlude the direct utility of CPUE methods. Line transect techniques are applicable to "sightable" species and require much thought to coverage definition. Hydroacoustic surveys are available for species that can be detected in the water column but can have problems with mixed species aggregations, murky water, and groundfish.

Mark-recapture studies are the primary technique for determining migration rates (Seber 1982). A recent approach utilizes a population dynamics model with maximum likelihood theory to estimate migration (Hilborn 1990; Heifetz and Fujioka 1991). Another recent approach is to use a diffusion approach to migration (Deriso et al. 1991). In some special circumstances, tag analysis can also be used to estimate abundance of a migratory population, as presented in Seber (1982, chapter 11). The technique requires temporal and/or spatial marking and recapturing but does not require constant probability of capture. This technique has been extended to the situation where a proportion of the marked population strays out of the region of interest (Eames et al. 1981). Major problems with mark-recapture estimation applied to commercial fisheries data are tag loss, lack of information on reporting rates, probability estimates that are less than 0 or greater than 1, and failure to meet the other stringent assumptions of mark-recapture methodology.

Migratory populations that pass a location can frequently be enumerated to estimate abundance, such as gray whales (Reilly 1981), bowhead whales (Zeh et al. 1986), and Pacific salmon (Mundy 1982, 1985). In the latter case, migratory timing curves are examined historically and used to provide in-season escapement estimates.

Variations of cohort analysis using catch-age data have been suggested to account for migration. Aldenberg (1975) and Ulltang (1977) developed a two-region migration procedure based on instantaneous migration rates. This procedure was used by Jones (1983) for North Sea saithe stocks. Cohort analysis was extended to account for migration information by Deriso and Quinn (1983), assuming that all migration occurs at the start of the year. To redistribute the population, a simple recurrence relationship was derived similar to cohort analysis of Pope (1972). This technique is useful for historical data, but a method is needed for current populations as well.

A simple procedure for estimating abundance for a migratory population with a commercial fishery is called catch-age analysis with CPUE partitioning. First the abundance of the entire population is determined with cohort or catch-age analysis, obviating the need to consider migration at this stage. Relative abundance for a region is estimated from the product of CPUE and regional area. Then regional abundance is the product of total population abundance and relative regional abundance (Quinn et al. 1982, 1985). In application to Pacific halibut this procedure was inaccurate, because catchability among regions varied over time (Quinn et al. 1985). Hence we were motivated to develop migratory catch-age analysis.

Migratory Catch-Age Analysis

Data Requirements

The data requirements for migratory catch-age analysis are fairly extensive. The population must be divided into geographic regions and data must be available for each region. Catch-age data must be available from several years, as in cohort analysis (Pope 1972; Hoag and MacNaughton 1978) and catch-age analysis with auxiliary information (Deriso et al. 1985). Auxiliary information should be available for each region, such as catch-effort, a spawner–recruit relationship, or survey estimates. Natural mortality is assumed to be known.

The major data requirement is information on migration rates among regions. The probable source of this information is mark-recapture estimates of migration rates (Seber 1982). For Pacific halibut, a procedure documented in the International Pacific Halibut Commission (IPHC) (1981) and Quinn et al. (1985) was used, similar to that of Ishii (1979), which examined relative tag recovery rates from several tagging experiments conducted over several years. Due to the lack of comprehensive tagging operations over all areas in a given year, it was not possible to estimate annual rates of migration. However, age-specific estimates of migration rates were determined by stratifying tag releases and recoveries by size (Table 1).

Over broad geographic regions, such information is difficult to acquire, due to problems of adequate coverage, obtaining reporting rates, tag loss, and determining capture probability. A sensitivity study is provided later to show the effect of migration rates on population estimates. In our development, we treat migration from the point of view of directional annual

TABLE 1. Migration rates from one area to another for different age groups of Pacific halibut.

To area (i)	From area (j)			
	1	2	3	4
Age 8				
1	0.9982	0.0200	0.0095	0.0099
2	0.0013	0.9768	0.0108	0.0210
3	0.0005	0.0032	0.9672	0.0743
4	0.0000	0.0000	0.0125	0.8948
Ages 9–11				
1	0.9961	0.0122	0.0061	0.0064
2	0.0029	0.9858	0.0070	0.0138
3	0.0010	0.0020	0.9788	0.0481
4	0.0000	0.0000	0.0081	0.9317
Ages 12–14				
1	0.9902	0.0095	0.0047	0.0049
2	0.0072	0.9890	0.0053	0.0103
3	0.0026	0.0015	0.9838	0.0366
4	0.0000	0.0000	0.0062	0.9482
Ages 15–20				
1	1.0000	0.0000	0.0000	0.0000
2	0.0000	1.0000	0.0000	0.0000
3	0.0000	0.0000	1.0000	0.0000
4	0.0000	0.0000	0.0000	1.0000

movement. Seasonal migration could also be incorporated for fisheries which occur throughout the year and have the necessary seasonal data. Some developments in estimating seasonal migration rates with mark-recapture data are given in Deriso et al. (1991).

Measurement Error Model

In this development, upper case letters are used for original parameters and variables. Lower case letters are used for corresponding logarithms. An underline (_) under a variable denotes a vector or a matrix. Let there be data from T years, A ages, and R regions, with corresponding subscripts t, a, and r.

This model is an extension of the measurement error model of Deriso et al. (1985), which details assumptions and equations relating catch and population dynamics. The model uses a forward recursion of abundance from starting values of abundance for all ages in the first year and the first age in all years. Thus there are $T + A - 1$ year-class parameters for each region.

For model simplicity, it is assumed that migration rates are known and occur instantaneously at the start of the year. Alternate formulations to model the migration process are conceivable: an instantaneous rate of migration occurring throughout the year analogous to instantaneous rates of fishing or natural mortality or a seasonal process occurring over a short time period. Our assumption was made to accommodate the available information on annual migration rates, which are based on summer releases and recoveries, and the character of the Pacific halibut fishery, which occurs during a short period in the summer.

Migration rates may depend on year, age, or year-class; in our application, they are a function of age. The $R \times R$ matrix of annual migration rates is denoted $\underline{\Theta}_a$, where the $(i, j)^{\text{th}}$ element of $\underline{\Theta}_a$, is the proportion of age a fish from region j that move to region i. The vector of abundance after migration $\underline{N}^*(t,a)$ is

$$(1)\quad \underline{N}^*(t,a) = \underline{\Theta}_a\, \underline{N}(t,a),$$

where $\underline{N}(t,a)$ is the vector of size $R \times 1$ containing the regional abundances $\{N(t,a,r),\ r = 1,\ldots,R\}$ at the start of the year t for age a. For a given region r, abundance at the start of the next year is then

$$N(t+1,a+1,r) = N^*(t,a,r)e^{-Z(t,a,r)},$$

where $Z(t,a,r) = F(t,a,r) + M(t,a,r)$, $F(t,a,r)$ is fishing mortality, and $M(t,a,r)$ is natural mortality. As natural mortality is difficult to estimate from catch-age data, we assume that natural mortality is known.

The catch predicted from the model is calculated from the Baranov catch equation

$$C(t,a,r) = \frac{F(t,a,r)}{Z(t,a,r)} N^*(t,a,r)\,(1 - e^{-Z(t,a,r)}),$$

for each age, year, and region. The observed catch $C'(t,a,r)$ is assumed to have a lognormal error structure with median $C(t,a,r)$. A lognormal distribution for a random variable is obtained from a normal distribution of the natural logarithm of that variable. The lognormal assumption is equivalent to assuming that the logarithm of observed catch is normally-distributed with expected value being the logarithm of predicted catch, because the median for a log-normal distribution is given by $\exp(\mu)$, where μ is the mean of the normal distribution.

In order to estimate parameters, fishing mortality is assumed to be separable into an age factor and a year factor for each region,

$$F(t,a,r) = S(a,r)G(t,r),$$

where age selectivity $S(a,r)$ is equal to 1 for at least one fully-recruited age, and $G(t,r)$ is full-recruitment fishing mortality. Let D be the number of ages for which selectivity parameters are to be estimated. This model assumes measurement error in catch but does not allow for process error in the catch equations. Specifically, the separability assumption is assumed to hold exactly in the catch process.

To incorporate auxiliary information, full-recruitment fishing mortality is related to fishing effort $E(t,r)$ as a lognormal variable with median $Q(r)E(t,r)$, where $Q(r)$ is catchability for region r. A straight-forward extension for fisheries with multiple gear types can be incorporated into this approach with separate parameters for selectivity, catchability, and fishing mortality for each gear type, as shown by Deriso et al. (1989) for a non-migratory population.

For each region the sum of squares criterion from Deriso et al. (1985) is, with logarithms of appropriate relations substituted as lower case symbols,

$$SSQ(r) = \sum_t \sum_a (c'(t,a,r) - c(t,a,r))^2 + \lambda \sum_t (g(t,r) - q(r) - e(t,r))^2,$$

where λ is a specified constant determining the influence of fishing effort. For each region there are $T + A - 1$ year-class parameters, D parameters $s(a,r)$, T parameters $g(t,r)$, and one parameter $q(r)$ to be estimated from $T \times A$ observations of catch and T observations of effort, using a nonlinear least squares procedure. It would be possible to have λ a function of r to implement different regional weightings, but in application, we simply use an equal weighting.

To implement this extended model, we modified our computer software program CAGEAN (©1988; see Deriso et al. 1985) slightly. We intercepted the model abundances at the start of the year and moved them according to the migration rates. We found it necessary to iterate sequentially among regions holding the other regions fixed, because the number of parameters was too large to attempt joint estimation of all parameters on our computer. Results of this ad hoc estimation procedure, described in Quinn et al. (1985), were not satisfactory. The results were sensitive to initial conditions and convergence to the lowest sum of squares could not be guaranteed. In order to do simultaneous estimation which we believed would correct some of these problems, we developed the process error model described in the next section.

Process Error Model

The process error model assumes that the catch is measured without error and that process error, or stochastic variation, occurs in the dynamics of the population. In this section, the process error model presented in Deriso et al. (1985) is extended for migration.

The model uses a backward recursion of the population from terminal abundances, using migration rates, catch, and natural mortality. We use a constant value of natural mortality M for simplicity of development, but the model is easily modified for variable natural mortality as a function of age, year, and/or area. The terminal abundances include all ages in the last year and the last age in all years. The recursion procedure is called migratory cohort analysis and was developed by Deriso and Quinn (1983). It assumes that migration occurs at the end of the year and that catch occurs in the middle of the year. First, the population dynamics equations are derived for a constant migration rate matrix Θ and then generalized for age-specific migration. Similar results can be obtained for migration rates as a function of year.

The vector of regional abundance at the start of the next year is

$$(2)\quad \underline{N}(t+1,a+1)=\underline{\Theta}[\underline{N}(t,a)e^{-M}-\underline{C}'(t,a)e^{-M/2}].$$

A recurrence relation is now written in closed form that expresses abundance at any time as a function of terminal abundances. From (2),

$$(3)\quad \underline{N}(t,a)=\underline{\Theta}^{-1}\underline{N}(t+1,a+1)e^{M}+\underline{C}'(t,a)e^{M/2}.$$

Equation (3) for abundance one year and age later is

$$(4)\quad \underline{N}(t+1,a+1)=\underline{\Theta}^{-1}\underline{N}(t+2,\ a+2)e^{M}+\underline{C}'(t+1,\ a+1)e^{M/2}.$$

Substituting (4) into (3) yields

$$(5)\quad \underline{N}(t,a)=\underline{\Theta}^{-2}\underline{N}(t+2,\ a+2)e^{2M}+\underline{\Theta}^{-1}\underline{C}'(t+1,\ a+1)e^{3M/2}+\underline{C}'(t,a)e^{M/2}.$$

To generalize (5) for an age-specific migration, the matrix $\underline{\Theta}_a$ is substituted for $\underline{\Theta}$ in (2) and (3), and the matrix $\underline{\Theta}_{a+1}$ is substituted for $\underline{\Theta}$ in (4), yielding

$$(6)\quad \underline{N}(t,a)=\underline{\Theta}_a^{-1}\underline{\Theta}_{a+1}^{-1}\underline{N}(t+2,\ a+2)e^{2M}+\underline{\Theta}_a^{-1}\underline{C}'(t+1,\ a+1)e^{3M/2}+\underline{C}'(t,a)e^{M/2}.$$

Repeating the recursion process (5) for non-age-specific migration for x years yields

$$(7)\quad \underline{N}(t,a)=\underline{\Theta}^{-x}\underline{N}(t+x,a+x)e^{xM}+\sum_{i=0}^{x-1}\underline{\Theta}^{-i}\underline{C}'\ (t+i,\ a+i)e^{M/2+iM},$$

where by definition $\underline{\Theta}^{-0}=\underline{I}$, the identity matrix. Equation (7) is exact because catch is assumed to be measured without error. Given terminal abundances $\underline{N}(T,a)$ for all a and $\underline{N}(t,A)$ for all t, any abundance $N(t,a,r)$ can be determined from (7) as

$$(8)\quad N(t,a,r)=\sum_{j=1}^{R}\theta_{rj}^{-I}N(t+I,\ a+I,j)e^{IM}+\sum_{i=0}^{I-1}\left[\sum_{j=1}^{R}\theta_{rj}^{-i}C'(t+i,\ a+i,j)\right]e^{M/2+iM}$$

where θ_{rj}^{-i} is the $(r,j)^{\text{th}}$ element of $\underline{\Theta}^{-i}$ and $I=\min(T-t,\ A-a)$. In practice, it is easier to use the recursive relationship (4) to generate abundances for a year-class, but we need (8) in order to calculate derivatives for our nonlinear least squares estimation algorithm, as explained below.

To generalize (8) for an age-specific migration rate matrix $\underline{\Theta}_a$, it follows from the development of (2) through (8) that the powers of the inverse of $\underline{\Theta}$ are replaced with the product of the inverses of age-specific migration matrices. It can be shown that the term $\underline{\Theta}^{-x}$ in (7) should be replaced with the matrix product

$$\underline{\Theta}_a^{-1}\cdots\underline{\Theta}_{a+x-1}^{-1},$$

and that the term $\underline{\Theta}^{-i}$ in (7) should be replaced with the matrix product

$$\underline{\Theta}_a^{-1}\cdots\underline{\Theta}_{a+i-1}^{-1}\text{ for } i>0$$

and should remain equal to 1 for $i=0$. The analog to Equation (8) follows by substituting the corresponding elements of the matrix products for the elements of $\underline{\Theta}^{-x}$ and $\underline{\Theta}^{-i}$.

Terminal abundances can be estimated only from additional model structure. It is assumed that the exploitation rate $U(t,a,r)$, the ratio of catch to abundance, is lognormally distributed with median $S(a,r)V(t,r)$. The term $S(a,r)$ is age selectivity as before, and $V(t,r)$ is full-recruitment exploitation rate. The process error approach uses a separability assumption for exploitation rate rather than for fishing mortality, as in the measurement error model, because this permits a tractable solution. For this model, the median catch is

$$(9)\quad C(t,a,r)=S(a,r)V(t,r)N(t,a,r).$$

Substituting (8) into (9) produces the model equation in terms of its parameters, which is then log-transformed for use in nonlinear least squares. The log transformation produces an almost-linear model

$$(10)\quad c(t,a,r)=s(a,r)+v(t,r)+n(t,a,r),$$

which simplifies the estimation process.

To incorporate auxiliary information, full-recruitment exploitation rate is related to fishing effort $E(t,r)$ as a lognormal variable with median $Q(r)E(t,r)$.

The appropriate sum of squares criterion from Deriso et al. (1985) extended over all regions is given by

$$(11)\quad SSQ=\sum_{r}\left[\sum_{t}\sum_{a}(c'(t,a,r)-c(t,a,r))^2+\lambda\sum_{t}(v(t,r)-q(r)-e(t,r))^2\right].$$

As before, there are $T\times A$ observations of catch and T obser-

vations of effort for each region r. The SSQ is minimized as a function of the terminal abundances for all regions, using nonlinear least squares.

The other parameters for each region r: D parameters $s(a,r)$, T parameters $v(t,r)$, and $q(r)$, are found directly, as shown below. Thus, it is possible to jointly estimate abundance for all regions, because the number of parameters to be estimated from nonlinear least squares has been reduced substantially from the measurement error model. By taking partial derivatives of SSQ in (11) with respect to the parameters and setting them equal to zero, as shown in Appendix I, the parameter estimates for each region are obtained as the solution of the following linear system of equations:

$$(12)\quad \underline{A}\,\underline{X} = \underline{B}.$$

The matrix $\underline{A}$ is composed of submatrices and can be written as

$$(13)\quad A = \begin{pmatrix} \underline{1}\ \{T\times D\} & (A+\lambda)\underline{I}\ \{\mathrm{T}\times\mathrm{T}\} & -\lambda\underline{1}\ \{T\times 1\} \\ T\underline{I}\ \{\mathrm{D}\times\mathrm{D}\} & \underline{I}\ \{D\times T\} & \underline{0}\ \{D\times 1\} \\ \underline{0}\ \{\times D\} & \underline{1}\ \{I\times T\} & -T\underline{1}\ \{1\times 1\} \end{pmatrix},$$

where $\underline{1}\{I\times J\}$ is an $I\times J$ matrix filled with ones,

$\underline{0}\{I\times J\}$ is an $I\times J$ matrix filled with zeros, and

$\underline{I}\{I\times I\}$ is the $I\times I$ identity matrix.

The vector $\underline{X}$ of size $D+T+1$ is composed of the $s(a,r)$, $v(t,r)$, and $q(r)$ for the given region, or

$$\underline{X} = \begin{pmatrix} \underline{s} \\ \underline{v} \\ \underline{q} \end{pmatrix}.$$

The vector B of size $D+T+1$ is

$$\underline{B} = \begin{pmatrix} \underline{B}_1 \\ \underline{B}_2 \\ \underline{B}_3 \end{pmatrix}.$$

where the t^{th} element of $\underline{B}_1$ is

$$(14)\quad B_{1t} = \sum_a (c'(t,a,r) - n(t,a,r)) + \lambda e(t,r),$$

the a^{th} element of $\underline{B}_2$ is

$$(15)\quad B_{2a} = \sum_t (c'(t,a,r) - n(t,a,r))$$

and the only element of $\underline{B}_3$ is

$$(16)\quad B_3 = \sum_t e(t,r).$$

The solution of the linear system (12) is simply

$$\underline{X} = \underline{A}^{-1}\underline{B}.$$

Thus, the process error model uses migratory cohort analysis to project abundance backward in time from terminal values of abundance, catch, migration rates, and natural mortality. Many nonlinear least squares routines require analytical derivatives of the dependent variable with respect to the parameters; these are derived in Appendix 2. The dependent variable in the nonlinear least squares algorithm is composed of the logarithm of catch for each time, age, and region from (10) and logarithm of auxiliary effort (multiplied by $\sqrt{\lambda}$) for each time and region. Independent variables are time, age, and region. Parameters estimated by the nonlinear least squares algorithm are logarithms of terminal abundances. The linear sytem of equations (12) is then solved for each region to estimate parameters $s(a,r)$, $v(t,r)$, and $q(r)$ within each iteration, allowing the computation of SSQ in (11). The terminal abundances are updated in subsequent iterations of the nonlinear least squares algorithm until convergence occurs.

Application

Commercial catch-age and effort data are available for Pacific halibut for four areas: Area 1 (International Pacific Halibut Commission Regulatory Areas 2A and 2B: British Columbia, Washington, Oregon, and California), Area 2 (Regulatory Area 2C: Southeast Alaska), Area 3 (Regulatory Area 3A: eastern Gulf of Alaska), and Area 4 (Regulatory Areas 3B and 4: western Gulf of Alaska and the Bering Sea); these areas encompass the entire geographic range of Pacific halibut. The range of ages is 8–20. Details regarding these data and their treatment are found in Quinn et al. (1985). These data are given in Appendix 3 to encourge other researchers to explore migratory methods. Fishing effort is measured in number of skates. Previous research was based on a J-hook standard; statistics in Appendix 3 are converted to a circle-hook standard which is now used by most of the fishing fleet. The time period of 1974–84 was chosen for comparison with other methods in Quinn et al. (1985). Natural mortality M is assumed to be 0.2 for all ages, years, and areas, in accord with other analyses (Quinn et al. 1985).

The value for the weighting coefficient λ of 2.0 is used in this paper. This value is selected to provide a moderate influence of the auxiliary effort information, so that the pattern of exploitable biomass is consistent with that of CPUE (Quinn et al. 1985). Other values of λ result in different estimates of population parameters, indicating that the choice of λ is critical. However, the value we use is probably of the correct order of magnitude, because λ is theoretically the ratio of variances of the logarithms of catch-age and effort data and empirical studies suggest that catch-age data for Pacific halibut is slightly more variable than effort data (Quinn et al. 1982, 1985). Other approaches for selecting λ are given in Deriso et al. (1989) and Kimura (1989).

Migration information, reported in Quinn et al. (1985), is reproduced in Table 1. Migration is highest for younger ages, by age 15 it is negligible. Migration is highest from Area 4 to other areas, but it is also notable from the other areas. No estimates of interannual or seasonal variation of migration rates are currently available.

Three analyses of the data were made. The first analysis was a closed-area analysis using the measurement-error approach of Deriso et al. (1985). Each area was analyzed separately as if migration did not occur. The second analysis was the process-error approach for migratory catch-age analysis presented in this paper using an identity matrix for migration. This analysis provides a contrast to the first analysis to examine the effect of the error structure on estimation. The third analysis was the process-error approach for migratory catch-age analysis using the migration information in Table 1. These analyses are contrasted to the measurement-error approach for migratory catch-age analysis from Quinn et al. (1985) in the Discussion section.

Parameters of interest include abundance, year-class strength (abundance at age 8), exploitable biomass (the sum of selectivity × abundance × average weight at age), and exploitable surplus production (the sum of yield and the change in exploit-

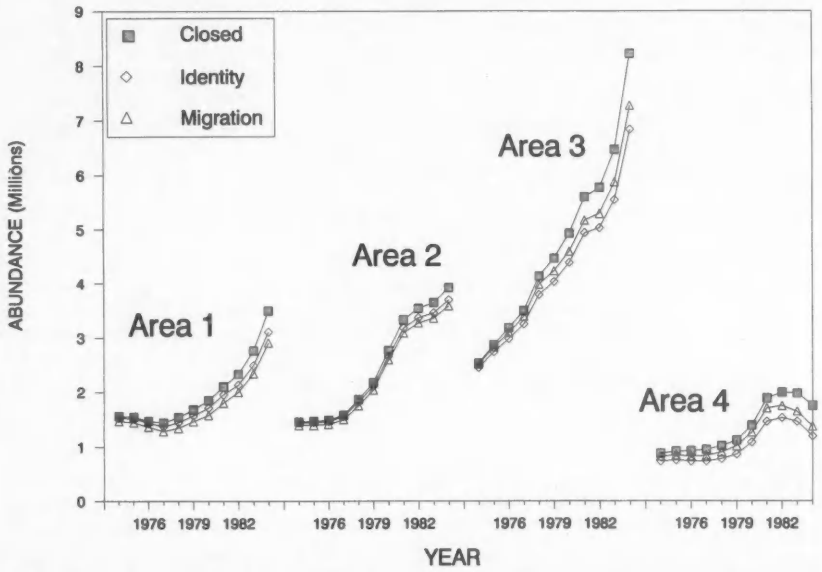

FIG. 1. Estimates of Pacific halibut abundance by area from 1974 to 1984 for three approaches: Closed area analysis (Closed), migratory catch-age analysis using an identity matrix for migration (Identity), and migratory catch-age analysis using migration estimates from Table 1 (Migration).

able biomass), in accord with previous analyses (Deriso et al. 1985; Quinn et al. 1985). In all analyses, the bootstrap method (Efron 1982) with 100 replications was used to assess variation and bias of parameter estimates. The bootstrap standard deviation is an estimate of the standard error of the parameter estimate. The difference between the bootstrap mean and the parameter estimate is an estimate of statistical bias (Efron 1982), as long as the model assumptions are satisfied.

Starting values for the first analysis were chosen from the automated routine found in our computer program CAGEAN (Deriso et al. 1985), which is a modified cohort analysis. Terminal fishing mortality to initiate the cohort analysis was 0.35. This consistent approach was not used in the analyses of Quinn et al. (1985), so closed-area results reported there are different from those found later. Starting values for the other two analyses were taken from the ending values of the first analysis. Starting values were varied occasionally to detect whether local minima were encountered. Using the closed area analysis to initiate starting values appeared to lessen this problem, resulting in the lowest sum of squares of the many variations attempted.

Results

In all analyses, convergence of the nonlinear least squares routine was rapid (under ten iterations). No difficulties in convergence occurred in the bootstrap replications either. For all parameters of interest, the estimated bias from the bootstrap was low compared to the standard deviation and is not considered further.

Estimated abundances of Pacific halibut summed over ages 8–20 are shown in Fig. 1 between 1974 and 1984 for each area for the three analyses. Estimated abundances are always higher for the first closed-area analysis than those from the two migratory catch-age analyses. As the second analysis used an identity matrix for migration and hence assumed no migration between areas, the difference among methods is attributed to the different error structures used. The first analysis is a measurement error model, while the second is a process error model. Nevertheless, the differences are not of a large magnitude, of the order 10% or less. Differences between the second and third analyses are due to use of the identity and actual migration matrices, respectively. Abundance is slightly lower in Areas 1 and 2 and slightly higher in Areas 3 and 4. Both migration rates and the estimates of terminal abundance affect these results; a sensitivity study later investigates these effects.

Coefficients of variation (the bootstrap standard deviation divided by the parameter estimate) increase from about 5 to 20% in Areas 1, 2, and 3 from 1974 to 1984 for all analyses, from 10 to 25% in Area 4 for the second and third analyses, and from 20 to 40% in Area 4 for the first analysis (Fig. 2). The relative precision of the estimates is lower for the most recent time period, as expected since those recent cohorts have not been sampled throughout their entire fishable life-span. The migratory analyses generally have lower coefficients of variation than the closed-area analysis. This finding could be a result of the larger sample size obtained by joint estimation of all areas or the fewer number of parameters per area that need to be estimated in the nonlinear least squares algorithm for the migratory catch-age analyses.

Trends in exploitable biomass estimates are quite similar to those in abundance (Fig. 3). Exploitable biomass incorporates selectivity and average weight as well as abundance into an overall quantity and as a result, differences among years and methods tend to be dampened slightly. Exploitable surplus production has further dampening of differences among methods (Fig. 4), because yield is a dominant component in its calculation. In addition, the annual change in exploitable biomass differs less among methods than exploitable biomass itself. Year-class strength, on the other hand, differs appreciably

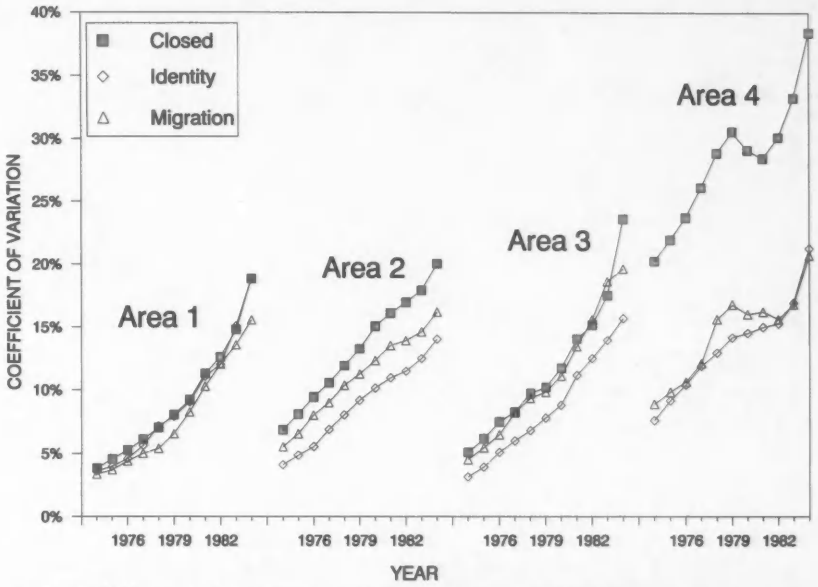

FIG. 2. Coefficients of variation (bootstrap standard deviation divided by the parameter estimate) for estimated Pacific halibut abundance by area from 1974 to 1984 for the Closed, Identity, and Migration approaches.

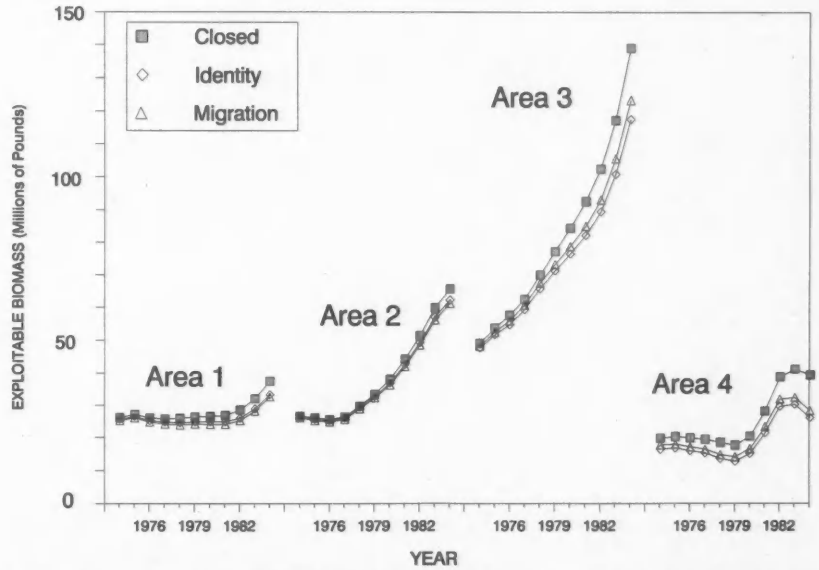

FIG. 3. Estimates of Pacific halibut exploitable biomass by area from 1974 to 1984 for the Closed, Identity, and Migration approaches.

among years, methods, and areas (Fig. 5), as expected since each one is a parameter for a single age in a single year in a single area. Standard deviations about these other parameters of interest show similar trends as those for abundance and are not reported here.

Analysis of residuals provides an indication of whether the statistical assumptions of the migratory model (the third analysis) are satisfied. Similar conclusions occur for the first two analyses. Notched box plots (Velleman and Hoaglin 1981) of residuals (on a logarithmic scale) as a function of area, age, and year show that the model fitted the data well; the area between the notches, indicating the 95% C.I. about the median residual for each factor level, generally contains the value 0 (Fig. 6). Significant non-zero medians do occur at older ages, suggesting the occurrence of ageing errors or non-constant selectivity, but the deviations are small. Outliers, while pres-

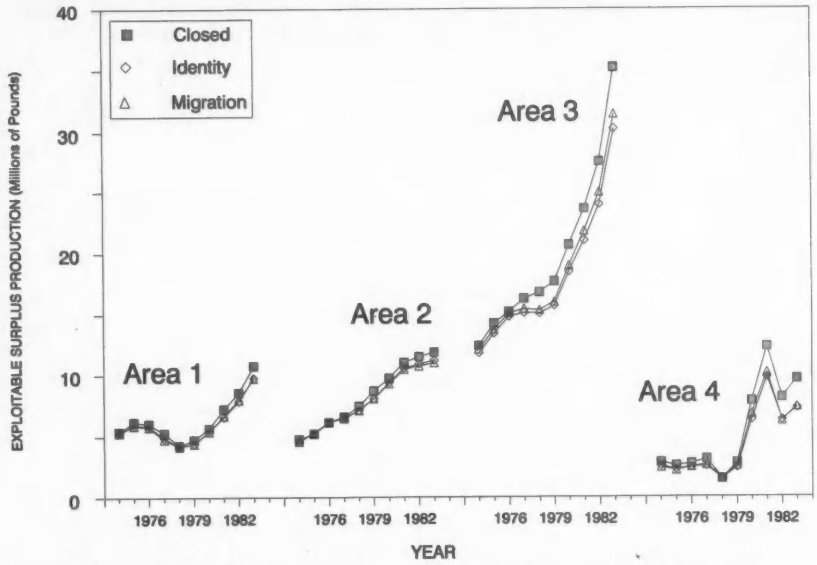

FIG. 4. Estimates of Pacific halibut exploitable surplus production by area from 1974 to 1984 for the Closed, Identity, and Migration approaches.

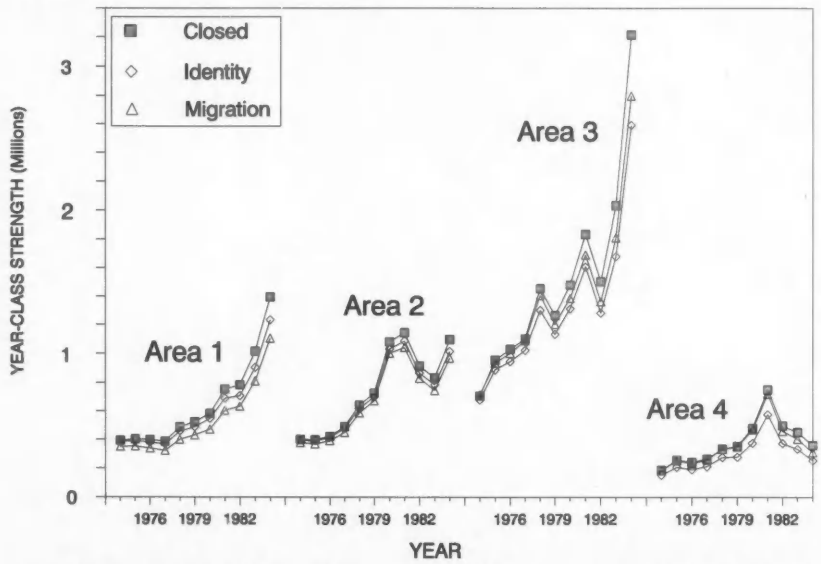

FIG. 5. Estimates of Pacific halibut yearclass strength at age 8 by area from 1974 to 1984 for the Closed, Identity, and Migration approaches.

ent, are not especially abundant, and occur evenly on positive and negative sides of the residuals axis. The similar shapes of the boxes suggest that the assumption of homoscedasticity is not unreasonable. Satisfaction of model assumptions is not surprising, considering the large number of parameters in the models.

Differences among the latter two analyses may be due to one of two factors: migration, or terminal values for abundance used to initiate the migratory cohort analysis process of the overall model. A sensitivity study of migratory cohort analysis was conducted to explore these factors. First, the standard values used were those obtained from the third analysis. The first perturbation was to double the terminal abundances, leaving migration the same. The second perturbation was to double migration rates for age 8 and use those for all ages, while leaving terminal abundances the same as standard values. This choice was made to obtain graphic clarity.

Results of the sensitivity study on migratory cohort analysis are shown in Fig. 7. Doubling the terminal abundances increases abundance across ages in all years and areas, with

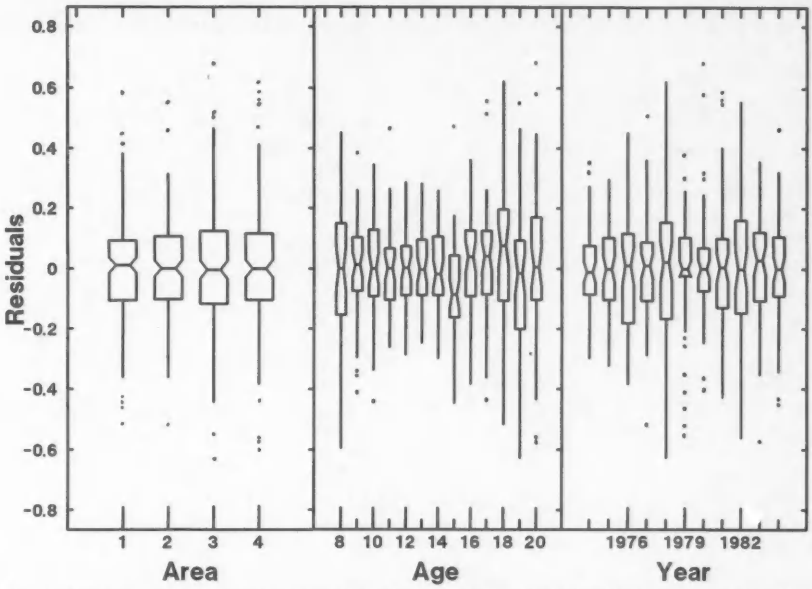

FIG. 6. Notched box plots of residuals on a logarithmic scale from the Migration approach as a function of area, age, and year. The notch corresponds to the width of the 95% C.I. about the median. The box gives the range of the middle 50% of the residuals. The lines indicate the range of the residuals, except for outliers (beyond 1.5 times the box length), which are plotted as individual points.

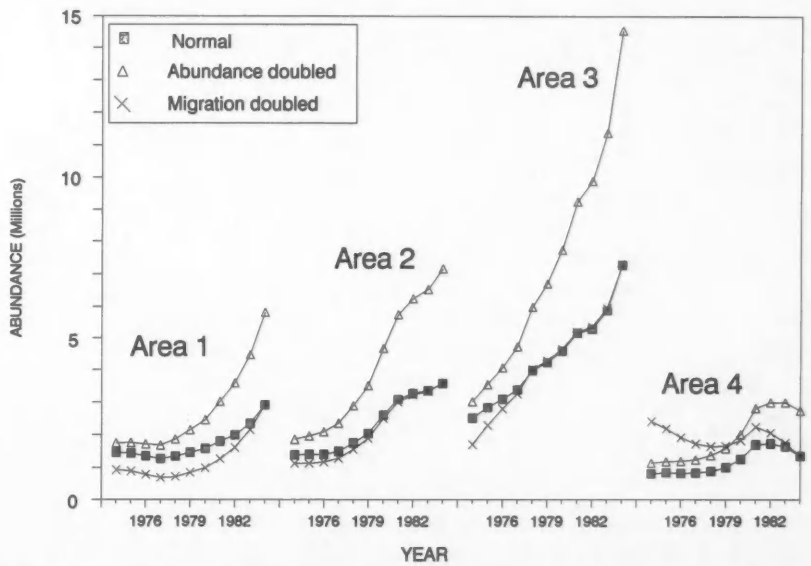

FIG. 7. Sensitivity of Pacific halibut abundance by area to starting values of abundance and migration using migratory cohort analysis. Estimates of abundance from the Migration approach are contrasted with those using doubled starting abundances and with those using doubled age 8 migration rates applied to all ages.

exactly double the effect in the last year and diminishing effect in each subsequent earlier year. This amelioration of the effect of terminal values occurs in one-area cohort analysis as well (Pope 1972).

The effect of migration is more complicated, depending on whether an area is a "source" or a "sink" or neither. In this example, Area 1 is clearly a sink, with migration mainly into this area and little out of it (Table 1). Area 4 is mainly a source with large migration out of the area. Areas 2 and 3 are neither,

having migration both in and out. In the second perturbation, abundance for earlier years in Areas 1, 2, and 3 is decreased and is increased in Area 4 (Fig. 7). The result of migration is to lower abundance in areas that are sinks and to increase abundance in areas that are sources as time proceeds backward. This redistribution of fish at earlier ages and times among areas is necessary to provide for the future migration of those fish into other areas. The effect of migration on migratory cohort analysis is similar to the effect of natural mortality in one-area cohort analysis. This result is not surprising, since migration out of an area can be considered a type of natural mortality of the population in that area.

Discussion

The models in this paper are generalizations of models found in the literature. For the special case of Θ being the identity matrix and the number of regions R being one, Eq. (2)–(8) are the generating equations for the familiar cohort analysis of Pope (1972). Under these same conditions, Eq. (9)–(11) are the same as those for the process error model of Deriso et al. (1985). One difference is that Deriso et al. (1985) used a forward-generating model for cohort analysis; this paper uses a backward-generating model. One advantage of the backward-generating model is that the number of parameters to be obtained by nonlinear least squares is reduced almost in half. One common problem in these migratory models is that negative estimates of abundance can occur if the redistribution of population due to migration is too severe. Such a problem would indicate an inconsistency in the data sources or a model deficiency.

The measurement error approach for migratory catch-age analysis was investigated in Quinn et al. (1985). There were problems in obtaining convergence of the nonlinear least squares solution, presumably due to the estimation procedure. Parameter estimates were obtained for one area given the estimates for the other areas. Then, iterations among areas were made until convergence was obtained. Given the ease of convergence in the process error model, it appears that joint estimation of all parameters is a better procedure. Joint estimation for the measurement error model is certainly possible, but the number of parameters to be estimated would require a large computer. Estimates of exploitable biomass from 1974 to 1984 from the measurement error model are 31–53 million pounds in Area 1, 25–74 million pounds in Area 2, 42–126 million pounds in Area 3, and 24–36 million pounds in Area 4 (Quinn et al. 1985, page 51). In comparison to the results in Fig. 3, only the Area 1 estimates differ greatly from these ranges. The difference is attributed to initial values used, because the closed-area estimates in that analysis show a similar range.

One limitation of the current approach is that migration rates are treated as constants rather than parameter estimates. Were estimates of standard error available for migration rates and an underlying distribution for the rates presumed, then a parametric bootstrap procedure (Efron 1982) could be used in conjunction with the regular bootstrap procedure. In the Pacific halibut application, the error structure in the model seemed to have greater effect than the migration rates, suggesting that estimates and standard errors would not change greatly if this source of variation were incorporated.

Migratory catch-age analysis and migratory cohort analysis are the best methods for assessment of the Pacific halibut population, according to the results of this study. Coefficients of variation were lower than those obtained for closed-area analysis and no appreciable statistical bias was detected. Parameter estimates of abundance were lower than those obtained for closed-area analysis, producing a more conservative outlook of the population.

One alternative to migratory catch-age analysis is to analyze the entire coastwide population using coastwide parameters for abundance, selectivity, exploitation, and catchability. One advantage to coastwide analysis is that coefficients of variation of age-composition and effort information are lower coastwide than for individual areas. However, assumptions about separability of age selectivity and exploitation rate are more likely to be satisfied on smaller geographic areas than the coast-wide Pacific halibut population. Management decisions are generally made separately for the different areas anyway, which requires partitioning estimates into areas in any case (Quinn et al. 1985). As trends in abundance (Fig. 1), exploitable biomass (Fig. 3), and year-class strength (Fig. 5) are different among areas, prudent management would require the best information for each area.

For other populations with migratory characteristics, the use of migratory catch-age analysis may be fruitful as well. Although the data requirements are more stringent, the assumptions of migratory catch-age analysis are less stringent. The recent development of methods for estimating migration rates from mark-recapture experiments (Hilborn 1990; Deriso et al. 1990; Heifetz and Fujioka 1990) suggests that a future research forefront will be the development of methodology which recognizes the migratory character of a population. The ultimate utility of migratory methods awaits reconcilement of the trade-off between the greater availability and higher precision of data with the failure of constant parameter assumptions over a large region.

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Appendix 1. Derivation of Estimates of Parameters $s(a)$, $v(t)$, and q for a Given Region

For clarity, the region subscripts have been dropped. To find parameter estimates, the partial derivatives of *SSQ* in (11) with respect to the parameters need to be derived and set equal to 0. Equation (10) is first substituted into (11) to obtain *SSQ* as a function of the parameters of interest. Only the *SSQ* for a given region has non-zero partial derivatives with respect to a given region's parameters $v(t)$, $s(a)$, and q.

With respect to a particular $v(t)$,

$$\frac{\partial SSQ}{\partial v(t)} = \sum_a s(a) + \sum_a n(t,a) + Av(t) - \sum_a c'(t,a) - \lambda[e(t)+q-v(t)] = 0,$$

$$\text{or } \sum_a s(a) + (A+\lambda)v(t) - \lambda q = \sum_a [c'(t,a) - n(t,a)] - \lambda e(t).$$

Note that for fully-recruited ages, $s(a) = 0$, so that summation over subscript a occurs with respect to the non-zero $s(a)$. With respect to a particular $s(a)$,

$$\frac{\partial SSQ}{\partial s(a)} = \sum_t v(t) + \sum_t n(t,a) + Ts(a) - \sum_t c'(t,a) = 0,$$

$$\text{or } Ts(a) + \sum_t v(t) = \sum_t [c'(t,a) - n(t,a)].$$

With respect to q,

$$\frac{\partial SSQ}{\partial q} = -\sum_t v(t) + \sum_t e(t) + Tq = 0,$$

$$\text{or } \sum_t v(t) - Tq = \sum_t e(t).$$

By writing these $D+T+1$ equations in $D+T+1$ unknown parameters in matrix form [equations (12) and (13) in text], it is clear that there is an exact solution.

Appendix 2. Derivatives of Catch with Respect to Abundance for a Nonlinear Least Squares Routine

Many nonlinear least squares routines require analytical formulae for the partial derivatives with respect to the parameters. For the process error model, the parameters are the logarithmic terminal abundances for all regions. From (10), the partial derivative of model catch in region r with respect to a given logarithmic terminal abundance $n(\tau,\alpha,x)$ in region x is

$$\text{(A2.1)} \quad \frac{\partial c(t,a,r)}{\partial n(\tau,\alpha,x)} = \frac{\partial s(a,r)}{\partial n(\tau,\alpha,x)} + \frac{\partial v(t,r)}{\partial n(\tau,\alpha,x)} + \frac{\partial n(t,a,r)}{\partial n(\tau,\alpha,x)},$$

where $\tau = t-I$, $\alpha = a-I$, and $I = \min(T-t, A-a)$. As shown in Appendix 1, $s(a,r)$ and $v(t,r)$ are solutions to the equation $\underline{X} = \underline{A}^{-1}\underline{B}$. For one of these parameters, say $X(i)$, this solution can be written

$$X(i) = \sum_j a(i,j)B(j),$$

where $a(i,j)$ is the $(i,j)^{\text{th}}$ element of $\underline{A}^{-1}$. Its derivative with respect to logarithmic terminal abundance is

$$\text{(A2.2)} \quad \frac{\partial X(i)}{\partial n(\tau,\alpha,x)} = \sum_j a(i,j)\frac{\partial B(j)}{\partial n(\tau,\alpha,x)},$$

because the terms of $\underline{A}^{-1}$ are not related to abundance. The term $\frac{\partial B(j)}{\partial N(\tau,\alpha,x)}$ is zero unless $\alpha - a = \tau - t$ and $\alpha \geq a$. In that case,

$$\text{(A2.3)} \quad \frac{\partial B(j)}{\partial n(\tau,\alpha,x)} = -\frac{\partial n(t,a,r)}{\partial n(\tau,\alpha,x)}$$

from the definition of $\underline{B}$ in (14) and (15) for $s(a,r)$ and $v(t,r)$.

To obtain $\frac{\partial n(t,a,r)}{\partial n(\tau,\alpha,r)}$, note that

$$\frac{\partial n(t,a,r)}{\partial n(\tau,\alpha,x)} = \frac{\partial \ln N(t,a,r)}{\partial \ln N(\tau,\alpha,x)} = \frac{N(\tau,\alpha,x)}{N(t,a,r)} \frac{\partial N(t,a,r)}{\partial N(\tau,\alpha,x)}.$$

From (8),

$$\frac{\partial N(t,a,r)}{\partial N(\tau,\alpha,x)} = \theta_{rx}^{-1} e^{MI}.$$

Hence,

$$\text{(A2.4)} \quad \frac{\partial n(t,a,r)}{\partial n(\tau,\alpha,x)} = -\frac{N(\tau,\alpha,x)}{N(t,a,r)} \theta_{rx}^{-1} e^{MI}.$$

Substituting (A2.2) and (A2.4) into (A2.1) yields the final solution, which can be generalized as in the model development for age-specific migration.

To incorporate auxiliary effort, define a dependent variable $Y'(t,r) = \sqrt{\lambda} e(t,r)$ and its model $Y(t,r) = \sqrt{\lambda}(v(t,r) - q(r))$. This construction models $v(t,r)$ as a normal random variable with expectation $q(r) + e(t,r)$ and allows the proper sum of squares to be constructed in (11). The partial derivative is

$$\text{(A2.5)} \quad \frac{\partial Y(t,a,r)}{\partial n(\tau,\alpha,x)} = \sqrt{\lambda} \left(\frac{\partial v(t,r)}{\partial n(\tau,\alpha,x)} - \frac{\partial q(r)}{\partial n(\tau,\alpha,x)} \right),$$

The first term on the right-hand side of (A2.5) is given above in (A2.2) and the second term is zero by examination of (16).

Appendix 3. Natural Logarithm of Pacific Halibut Catch by Area, Year, and Age and Natural Logarithm of Fishing Effort by Area and Year

Area 1	Age													Log
Year	8	9	10	11	12	13	14	15	16	17	18	19	20	effort
1974	9.930	10.037	9.964	9.723	9.394	9.625	8.653	8.280	8.813	8.091	7.830	7.422	7.358	10.512
1975	9.896	10.250	10.396	10.398	10.278	9.558	9.697	8.675	8.722	8.628	8.312	7.618	7.885	10.848
1976	10.495	10.343	10.460	10.224	10.079	9.389	9.134	9.089	7.859	7.919	7.472	7.146	6.418	11.094
1977	10.058	9.939	10.048	9.844	9.780	9.484	9.136	8.619	8.695	7.617	7.255	6.681	6.838	10.628
1978	9.934	9.854	9.631	9.573	9.486	8.897	8.917	8.496	8.076	7.509	7.246	6.880	7.137	10.449
1979	10.070	10.198	9.844	9.726	9.690	9.239	8.996	8.588	8.353	7.661	6.984	6.273	6.555	10.744
1980	10.071	10.269	10.175	10.007	9.712	9.432	9.056	9.002	8.751	8.289	7.971	7.339	7.272	10.587
1981	10.359	10.589	10.356	10.171	9.746	9.387	9.314	8.782	8.459	8.119	7.848	7.030	6.743	10.352
1982	10.285	10.524	10.402	10.010	9.809	9.382	8.948	8.453	8.222	7.785	7.661	7.193	6.488	10.423
1983	10.328	10.447	10.550	10.148	9.862	9.310	9.019	8.712	8.332	7.488	7.799	7.031	6.646	10.402
1984	11.179	11.341	11.031	10.774	10.202	9.791	9.540	8.382	8.466	8.167	7.541	7.082	6.828	10.846

Area 2	Age													Log
Year	8	9	10	11	12	13	14	15	16	17	18	19	20	effort
1974	9.345	9.782	9.717	9.823	9.598	9.871	9.037	9.061	9.029	8.551	7.844	7.583	7.272	10.703
1975	9.749	9.963	9.947	9.839	9.817	9.450	9.569	8.837	8.788	8.570	8.172	7.394	7.233	10.881
1976	9.517	9.831	9.858	9.651	9.633	9.473	9.162	9.428	8.519	8.458	8.054	7.469	6.930	10.995
1977	8.891	9.390	9.510	9.345	9.109	8.937	8.649	8.470	8.280	7.518	7.188	6.876	6.089	10.375
1978	9.363	9.606	9.756	9.503	9.458	9.137	8.802	8.461	7.854	7.465	6.664	6.520	6.139	10.457
1979	9.135	9.697	9.683	9.669	9.562	9.204	8.891	8.489	7.993	7.672	6.764	6.439	6.541	10.152
1980	9.767	9.620	9.832	9.411	9.286	8.919	8.674	8.143	7.834	7.402	7.334	6.918	6.399	9.827
1981	9.650	10.098	9.804	9.734	9.454	8.856	8.550	8.286	8.181	7.342	7.701	6.503	6.727	9.930
1982	8.797	9.608	9.793	9.654	9.280	8.951	8.333	7.957	8.035	7.341	7.360	7.236	6.188	9.417
1983	9.301	10.044	10.404	10.260	10.003	9.591	9.346	8.910	8.324	8.251	7.870	6.676	7.078	10.090
1984	9.455	9.796	10.173	10.295	10.056	9.648	9.203	8.685	8.272	8.149	7.654	6.953	6.103	10.012

Area 3	Age													Log
Year	8	9	10	11	12	13	14	15	16	17	18	19	20	effort
1974	9.519	9.658	10.112	10.204	10.041	10.274	9.158	9.021	9.242	8.236	7.705	7.742	5.670	10.960
1975	10.129	10.400	10.326	10.425	10.385	9.996	10.219	9.057	9.017	8.582	7.807	7.172	6.974	11.198
1976	10.277	10.598	10.473	10.243	10.266	10.017	9.866	9.680	8,496	8.241	8.214	7.584	6.941	11.339
1977	9.607	10.452	10.394	9.946	10.003	9.897	9.551	9.360	9.189	8.210	8.022	7.188	6.605	11.070
1978	10.411	10.353	10.586	10.398	10.183	9.768	9.647	9.219	9.070	8.113	7.428	6.166	6.883	11.000
1979	10.190	10.634	10.750	10.535	10.431	9.853	9.749	9.195	8.993	8.216	7.820	6.602	6.715	11.002
1980	10.258	10.332	10.839	10.735	10.575	10.291	10.112	9.420	9.257	8.649	8.418	8.163	7.694	10.735
1981	10.208	10.721	10.678	10.957	10.719	10.520	10.077	9.597	9.165	8.916	8.505	7.600	8.015	10.944
1982	9.579	10.342	10.533	10.580	10.766	10.487	10.237	9.616	9.061	9.175	8.063	8.029	7.376	10.805
1983	10.097	10.585	11.065	10.763	10.775	10.346	9.814	9.552	9.431	8.752	8.045	7.749	7.092	10.705
1984	10.725	10.928	11.100	11.513	11.035	10.459	10.376	10.003	9.049	9.036	7.883	7.638	6.629	10.786

Area 4	Age													Log
Year	8	9	10	11	12	13	14	15	16	17	18	19	20	effort
1974	8.703	8.821	8.956	9.078	8.899	8.901	7.832	7.955	7.867	7.404	6.510	6.827	6.555	9.711
1975	9.244	9.211	9.420	8.936	9.155	8.683	9.007	7.780	7.816	7.836	7.387	6.675	6.819	9.918
1976	9.473	9.821	9.276	9.125	8.910	8.641	8.349	8.467	7.492	7.684	7.304	7.120	6.708	10.052
1977	9.366	9.618	9.934	9.293	9.314	9.032	8.932	8.400	8.828	7.826	7.538	7.139	6.669	10.194
1978	8.727	9.003	8.849	8.821	8.391	8.196	8.127	8.237	8.026	7.930	7.618	7.108	7.000	9.877
1979	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
1980	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
1981	8.879	8.724	8.580	8.552	8.284	7.914	7.680	7.130	6.872	7.119	6.544	6.467	5.913	8.766
1982	9.562	10.439	10.343	9.856	9.799	9.139	8.579	8.262	7.353	7.066	6.811	6.011	5.850	9.980
1983	10.254	10.665	11.237	10.613	10.251	10.133	9.576	8.989	8.417	8.082	7.344	7.122	6.320	10.663
1984	9.923	10.343	10.465	10.761	10.155	9.644	9.490	8.887	8.476	8.071	7.537	7.461	7.412	10.384

0.000 denotes missing values.

A Comparison of Methods for Assessment of Nutrient Deficiency of Phytoplankton in a Large Oligotrophic Lake

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Dodds, W. K., and J. C. Priscu. 1990. A comparison of methods for assessment of nutrient deficiency of phytoplankton in a large oligotrophic lake. Can. J. Fish. Aquat. Sci. 47: 2328–2338.

Short-term (h) and long-term (d) changes in phytoplankton community physiology and biomass in response to nutrient enrichment were used concomitantly as bioassays of phytoplankton nutrient deficiency in oligotrophic Flathead Lake, Montana, six times over the course of a year. Long-term bioassays consisted of nutrient amendments to epilimnetic water in 20 L containers which were subsequently monitored for algal growth. Short-term bioassays included measurement of NH_4^+ stimulation of dark carbon fixation, measurement of PO_4^{3-} and NH_4^+ uptake over time to assess depletion of internal pools and stimulation effects of PO_4^{3-} on NH_4^+ uptake and NH_4^+ on PO_4^{3-} uptake. During thermal stratification, simultaneous additions of NH_4^+ and PO_4^{3-} in long-term bioassays caused significant increases in chlorophyll *a* concentration, photosynthetic $^{14}CO_2$ uptake, and particulate N concentration within 4.5 d; single additions of NH_4^+ or PO_4^{3-} had little or no effect. During winter mixing there was little evidence for N or P deficiency in either short- or long-term bioassays. In general, short-term bioassays did not consistently agree with each other or with long-term bioassays. Our results suggest that it may be necessary to elicit growth of phytoplankton with nutrient addition to make definitive statements regarding nutrient deficiency.

Des modifications à court terme (h) et à long terme (j) de la physiologie et de la biomasse au sein de communautés phytoplanctoniques ont été induites de façon concomitante par enrichissement en éléments nutritifs à titre d'essais biologiques pour évaluer les carences alimentaires du phytoplancton dans le lac Flathead, un lac oligotrophe du Montana. Cette expérience a été répétée six fois au cours de la même année. Pour les essais à long terme, de l'eau de l'épilimnion a été enriche dans des contenants de 20 L mis sous observation pour y suivre la croissance des algues. Plusieurs types d'essais à court terme ont été effectués : mesure de l'effet stimulant du NH_4^+ sur la fixation du carbone durant la phase obscure, de l'absorption du PO_4^{3-} et du NH_4^+ dans le temps pour évaluer la diminution des réserves internes, et de l'effet stimulant du PO_4^{3-} sur l'absorption du NH_4^+ et réciproquement. Durant la période de stratification thermique, l'addition simultanée de NH_4^+ et de PO_4^{3-} dans les essais à long terme a causé, en 4, 5 j, des augmentations significatives de la concentration de la chlorophylle *a*, de l'absorption photosynthétique de $^{14}CO_2$ et de la concentration de N particulaire; ajoutés individuellement, ces deux éléments nutritifs avaient peu ou pas d'effet. Durant la période de mélange des eaux en hiver, les deux types d'essais biologiques tendaient légèrement à montrer l'existence d'une carence en N ou en P. De façon générale, les résultats des essais à court terme ne coïncidaient pas toujours d'essais en essais ou avec ceux des essais à long terme. Nos résultats laissent entendre qu'il pourrait s'avérer nécessaire de stimuler la croissance du phytoplancton par l'addition de nutriments pour pouvoir se prononcer de façon définitive sur les carences nutritives du phytoplancton.

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Algal biomass in oligotrophic lakes is often controlled by nutrient supply. Accurate information about nutrient deficiency and algal growth response to nutrient addition is useful to both lake managers and aquatic scientists. Numerous bioassays have been proposed for determining nutrient deficiency in natural phytoplankton assemblages. Stimulation of photosynthetic $^{14}CO_2$ uptake following nutrient enrichment is perhaps the most commonly used bioassay to assess N or P deficiency. Other bioassays for N or P deficiency based on short-term changes (h) in uptake rates of NH_4^+ or PO_4^{3-} or based on NH_4^+ enhancement of dark carbon fixation have been employed, but do not provide incontrovertible results (e.g. White et al. 1985; Vincent 1981a, b). Short-term bioassays can be convenient and cost effective and would be extremely useful if they provided reliable results. Unfortunately, results from short-term bioassays are often not compared to long-term bioassays which are based on net increases in the biomass of the phytoplankton community (Hecky and Kilham 1988). The large number of techniques proposed for the determination of nutrient deficiency and the lack of consensus on which technique is the most reliable, suggests the need for comparison of techniques in a single system.

We applied bioassays for N and P deficiency based on short-term (h) NH_4^+ and PO_4^{3-} uptake response and long-term (d) growth response of the phytoplankton, six times over the annual cycle in an oligotrophic lake. In the following section, we explain the rationale behind the various bioassays used. Results from these bioassays were contrasted to assess their relative utility for determination of phytoplankton N and P deficiencies.

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Rationale for the Nutrient Bioassays Employed in this Study

Biochemical Composition of Suspended Particulate Matter

Molar ratios of C:N:P in algal cells reflect the N:P supply ratio under which they were grown (Healey 1975; Redfield 1958; Suttle and Harrison 1988). Therefore, the C, N, and P content of particulate matter could reflect nutrient supply ratios and thus nutrient deficiencies in natural phytoplankton assemblages. We collected data on C, N, and P content of particulate matter in the epilimnion throughout the year, corrected for non-algal particulate matter using the regression procedure outlined in Priscu and Goldman (1983), and employed deviations from the Redfield (1958) ratio to infer nutrient deficiency.

Growth Response to Nutrient Enrichment

Long-term measurements of biomass and photosynthesis ($^{14}CO_2$ uptake) of natural phytoplankton were used to gauge response to nutrient enrichment. Containment effects (Hecky and Kilham 1988) and spurious short-term effects (Axler and Goldman 1981; Healey 1979; Lean and Pick 1981) can complicate the use of $^{14}CO_2$ uptake for gauging nutrient response. For these reasons, we enriched large (20 L) carboys containing lake water with nutrients, incubated the carboys in situ (at 4 m) to maintain the natural temperature and light regime, and monitored the growth of the algae over several days.

NH_4^+ Enhancement of Dark Carbon Fixation

Stimulation of dark carbon fixation following NH_4^+ addition has been proposed by Morris et al. (1971) as a short-term physiological assay to indicate N deficiency. This assay is based upon the observation that NH_4^+ enrichment of N deficient unialgal phytoplankton cultures causes an increase in dark carbon fixation by stimulating anaplerotic carboxylation activity. Despite the fact that this assay does not always correspond to other bioassays of natural phytoplankton populations (Axler and Goldman 1981; Vincent 1981*a*), it is often used to indicate N deficiency (e.g. Elser et al. 1988; Groeger and Kimmel 1988; Lean et al. 1990; St. Amand et al. 1989; White et al. 1985).

Stimulation of V_{max}

The maximum uptake velocity (V_{max}) of a non-limiting nutrient can be regulated by the availability of a limiting nutrient. Addition of PO_4^{3-} was shown to stimulate saturated uptake of dissolved inorganic N in a P-deficient oligotrophic lake (Dodds and Priscu 1990; Whalen and Alexander 1984), but not in a P-sufficient eutrophic reservoir (Toetz 1981). Conversely, under N deficiency the addition of N should stimulate P uptake. This hypothesis was tested in Flathead Lake by enriching aliquots of lake water with PO_4^{3-} or NH_4^+ for 12 h and then measuring $^{15}NH_4^+$ or $^{32}PO_4^{3-}$ uptake, respectively. Rates in enriched samples were compared to controls.

Internal Pools

Depletion of an intracellular nutrient pool often implies deficiency (e.g. Dortch 1982). An immediate lowering of the initial rate of uptake of a specific nutrient caused by exposure to saturating concentrations of that nutrient implies that internal pools were depleted (Healey 1975). We examined the effect of 12-h prior exposure of the natural phytoplankton community to NH_4Cl or KH_2PO_4 on $^{15}NH_4^+$ or $^{32}PO_4^{3-}$ uptake, respectively, to detect depletion in intracellular pools. This assay approximates the luxury consumption assays for nutrient deficiencies used by Vincent (1981a,b) and White et al. (1985) which examined short-term changes in particulate N (PN) and particulate P (PP) concentrations as indications of N and P uptake. However, our approach should not be sensitive to high background levels of non-algal PN and PP because incorporation was measured by direct isotopic incorporation.

End Products of Photosynthesis

Nitrogen deficiency should cause a decrease in phytoplankton protein synthesis because N is an essential metabolic precursor for this process. Priscu and Priscu (1984a) observed a significant increase in $^{14}CO_2$ incorporation into protein following NH_4^+ enrichment in a N deficient phytoplankton community. This result led them to suggest that such an assay might be used to detect N deficiency. Following their approach, we compared photosynthetic $^{14}CO_2$ incorporation into protein in unamended lake water and NH_4^+-enriched lake water. We also examined the effect of PO_4^{3-} enrichment on the apportioning of $^{14}CO_2$ incorporation into the end-products of photosynthesis to determine if consistent patterns emerged.

Materials and Methods

Study Site and Common Methods

Experiments were conducted on Flathead Lake, a 460 km^2 lake in northwestern Montana (Potter and Stanford 1975). Baseline conditions for the epilimnion of this oligotrophic lake are presented in Table 1. Water was collected from 5 m, approximately the zone of maximum photosynthesis, with a displacement sampler (Dodds and Priscu 1988) in the deepest part (100 m) of the lake.

All bioassays were conducted in June, July, August, November, February, and May except the long-term bioassays which were not conducted in July. June, July, and August experiments took place during thermal stratification, November experiments at the onset of winter mixing, and February and May experiments during mixing.

Phaeophytin-corrected chlorophyll *a* (chl *a*) retained on Whatman GF/F filters was measured fluorometrically (Strickland and Parsons 1972). NH_4^+, $NO_3^- + NO_2^-$, and soluble reactive phosphorus (SRP) were determined on 10 mL samples by the phenol-hypochlorite (Solorzano 1969), cadmium reduction (Eppley 1978), a phospho-molybdate (Strickland and Parsons 1972) methods respectively. Particulate P was determined by dry oxidation (Solorzano and Sharp 1980) and PC and PN with a Carlo-Erba 1106 elemental analyzer after filtration onto pre-combusted Whatman GF/F filters.

Long-Term Carboy Bioassays

Water was collected between 16:00 and 20:00, mixed in 100 L polyethylene-lined containers, and siphoned into 20 L collapsible, translucent polyethylene carboys. Care was taken during filling and subsequent sampling not to expose water to surface light which may cause long-term inhibition of phytoplankton. June and August bioassays were prefiltered through 280 μm mesh before nutrient addition to remove zooplankton. Zooplankton were no longer removed after it was determined

TABLE 1. Characteristics of 5 m water in Flathead Lake for selected dates. SRP = soluble reactive phosphorus. ND = no data.

Date	Particulates			Chl *a*	Nutrients		
	μM C	μM N	μM P	(μg·L^{-1})	μM NO_3^-	μM NH_4^+	μM SRP
11 July 1987	31.2	1.7	0.06	0.18	0.09	0.57	0.03
20 July 1987	26.3	1.9	0.07	0.51	ND	0.66	0.06
10 Aug. 1987	14.8	1.1	0.05	0.15	0.03	ND	0.05
6 Nov. 1987	31.3	2.4	0.07	0.48	0.06	0.49	0.02
3 Feb. 1988	21.0	2.2	0.10	0.97	3.26	0.82	0.02
2 May 1988	49.5	3.2	0.19	0.60	0.86	0.88	0.01

that ambient levels of zooplankton did not effect the outcome of the bioassays (Spencer and Ellis 1990). Carboys were enriched with 0.2 μM KH_2PO_4 (low P), 1.0 μM NH_4Cl (low N), 2 μM KH_2PO_4 (high P), 10 μM NH_4Cl (high N), 10 μM $NaNO_3$, 10 μM $NaNO_3$ + 1 nM Na_2Mo_4, 1/10 strength micronutrient solution (Castenholz 1981) and various combinations of the above. The 10 μM NH_4^+ and 2 μM PO_4^{3-} additions were sufficient to saturate uptake (unpubl. data). Two, 20 L carboys were used for each treatment in June and August and three carboys per treatment in November, February, and May. Carboys were suspended in the lake at 4 m to simulate light and temperature at the depth of collection.

The rate of photosynthetic [^{14}C]-$NaHCO_3$ incorporation was determined from 10:00 to 14:00 each day on 100 mL aliquots removed from all carboys. Isotope was added to a final concentration of 407 Bq·mL^{-1}, and aliquots were incubated at 5 m from 10:00 to 14:00. The samples were then filtered onto Whatman GF/F filters, washed five times with 5 mL water, and counted by standard liquid scintillation spectrometry. Aliquots were also removed daily from the carboys for determination of water chemistry, PP, PN, and chl *a* concentration.

Dark Carbon Fixation

Dark carbon fixation was measured on three control (unenriched) and three samples (100 mL each) enriched to a concentration of 10 μM NH_4Cl 1 h before $^{14}CO_2$ was added. [^{14}C]-$NaHCO_3$ was then added to each sample to a final concentration of 24.4 kBq·mL^{-1}. Following 8 h of dark incubation at lake temperature, the samples were filtered under reduced light onto Whatman GF/F filters and counted as above.

Stimulation of V_{max}

Experiments to examine stimulation of NH_4^+ uptake after 12 h of PO_4^{3-} enrichment at V_{max} were conducted in six, 500 mL bottles of pre-screened lake water collected between 16:00 and 20:00. Half of the bottles were enriched with 2 μM KH_2PO_4 and all six bottles were incubated in the dark at 20°C. After 12 h, 2 μM KH_2PO_4 was added to the three unenriched bottles, and 10 μM of 99 atom % [^{15}N]-NH_4Cl was added to all bottles which were then incubated for 6 h at 5 m in the lake. The samples were filtered on precombusted Whatman GF/F filters which were subsequently analyzed for ^{15}N enrichment by optical emission spectrometry (Timperley and Priscu 1986) following Dumas combustion (Fiedler and Proksch 1975). Particulate N was determined on a subsample of each filter. We did not observe significant changes in PN or SRP levels during the 12 h pre-incubation. This design allowed us to examine the effect of P addition on N uptake.

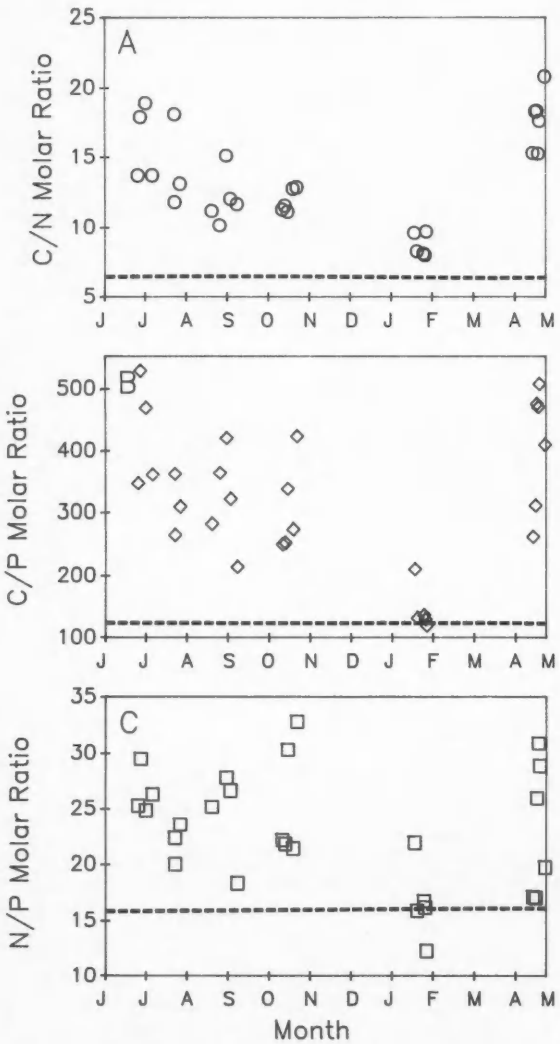

FIG. 1. Molar ratios of ambient PC:PN (A), PC:PP (B), and PN:PP (C) in Flathead Lake. Data are from water collected at 5 m between June 1987 and May 1988. Dashed lines represent Redfield ratios.

Stimulation of saturated PO_4^{3-} uptake by NH_4^+ was measured in eight, 100 mL bottles of 280 μm-screened lake water which were filled at 18:00. Four bottles were immediately

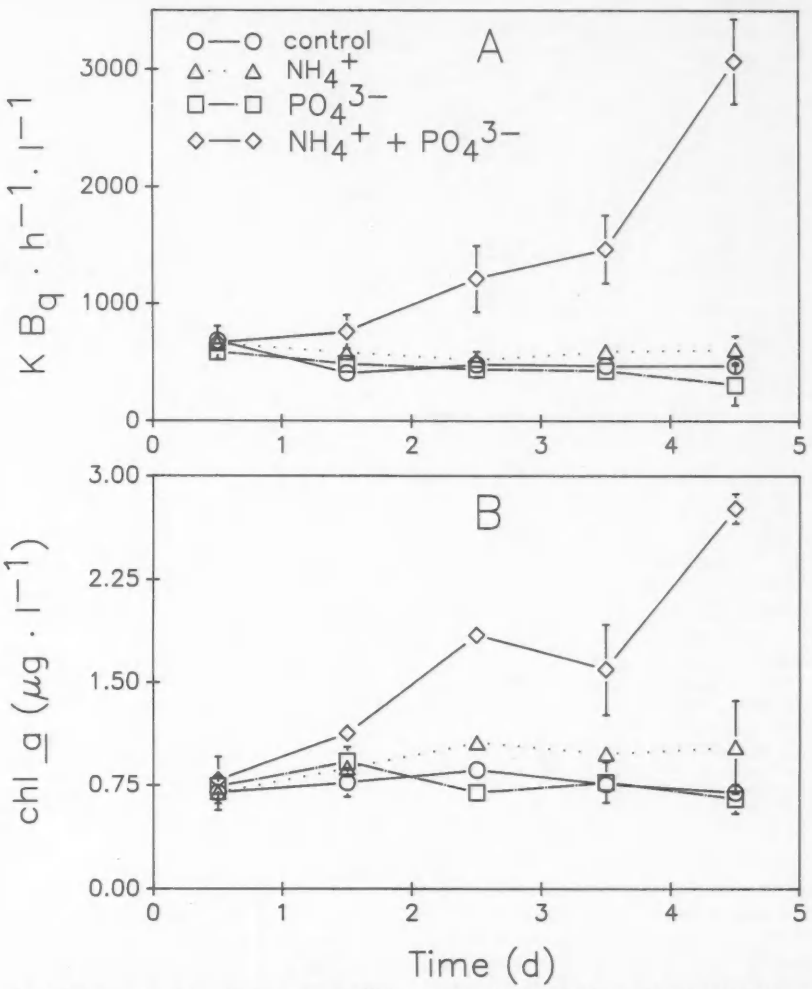

FIG. 2. Time-course of nutrient response of $^{14}CO_2$ uptake and chlorophyll *a* on 11 July 1987. Measurements were made on aliquots from 10:00 to 14:00 on each day. (A) $^{14}CO_2$ incorporation; (B) Phaeophytin corrected chl *a*.

enriched with 10 μM NH_4Cl and all eight bottles were incubated for 12 h in the dark at 20°C. After 12 h, 10 μM NH_4Cl was added to the four unenriched bottles. All bottles were then enriched with 2 μM KH_2PO_4 and 666 Bq·mL^{-1} carrier free [^{32}P]-H_3PO_4. Samples were incubated for 1 h in situ and then filtered on Gelman GN-6 filters (0.45 μm retention). Blank filters were placed beneath the sample filters to correct for label adsorbed on filters. Formalin-poisoned controls were used to correct for abiotic labeling in the particulate fraction. Gelman GN-6 filters showed the same retention of ^{32}P labeled particulates as Whatman GF/F filters ($P>0.10$, $n=6$, *t*-test). Filters were counted by liquid scintillation spectrometry.

Depletion of Intracellular Pools

Prescreened (280 μm) lake water was collected and decanted into six, 500 mL glass bottles between 16:00 and 20:00; three bottles were enriched with 5 μM NH_4Cl and all bottles were incubated in the dark for 12 h at 20°C. After 12 h, 5 μM NH_4Cl was added to the three unenriched bottles and 5 μM, 99 atom % [^{15}N]-NH_4Cl was added to all bottles. After 6 h of incubation at 5 m in the lake, the samples were filtered and analyzed for ^{15}N enrichment as described above.

Water for corollary P uptake experiments was collected during the early evening and decanted into eight, 100 mL bottles. Four of these bottles were enriched with 2 μM KH_2PO_4 and all eight bottles were incubated for 12 h at 20°C in the dark. After 12 h, 2 μM KH_2PO_4 was added to the four unenriched bottles and 666 Bq·mL^{-1} of carrier free [^{32}P]-H_3PO_4 were added to all samples. Following 1 h of incubation, ^{32}P incorporation was determined as discussed previously.

End Products of Photosynthesis

Triplicate lake water samples were amended with 10 μM NH_4Cl or 2 μM KH_2PO_4; controls remained unamended. [^{14}C]-

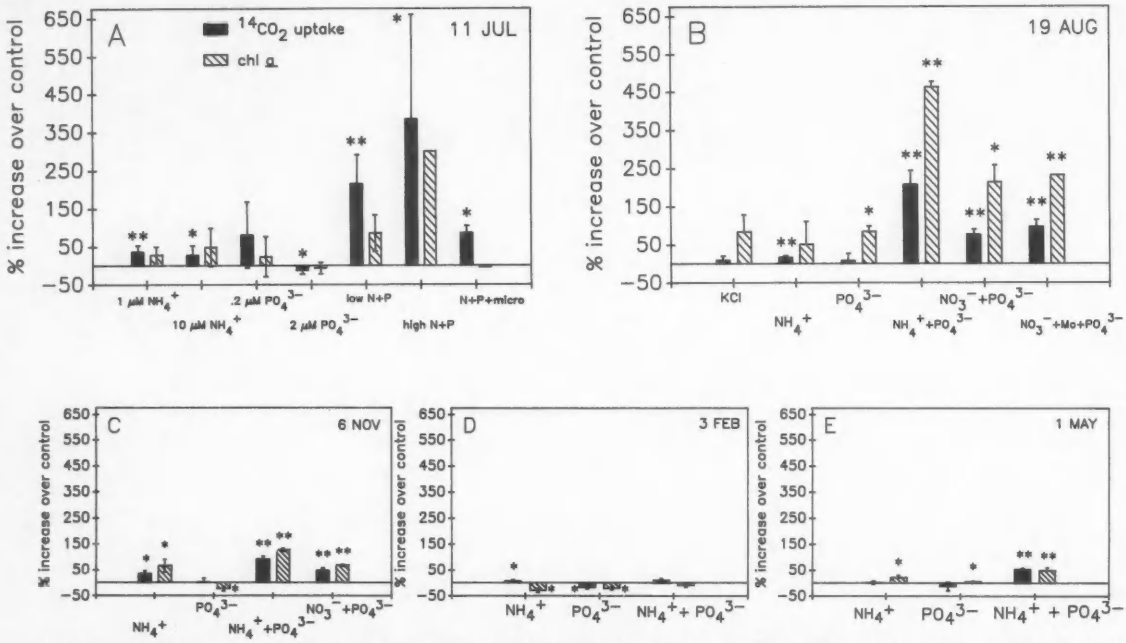

FIG. 3. Change in chlorophyll *a* and $^{14}CO_2$ uptake compared to unfertilized carboys after 4.5 d for five times during the year in Flathead Lake. Solid bars represent the percent increase over control in $^{14}CO_2$ uptake, crosshatched bars represent percent increase in chlorophyll *a*. Error bars represent 1 SD. Treatments which are significantly different than the control are denoted by * ($P<0.05$) or **($P<0.01$). Unless otherwise indicated 10 μM N and 2 μM PO_4^{3-} were added. (A) low N = 1 μM NH_4Cl, high N = 10 μM NH_4Cl, low P = 0.2 μM PO_4^{3-}, high P = 2 μM PO_4^{3-}. (19 Aug. data (B) for percent increase in $^{14}CO_2$ uptake with NH_4^+, PO_4^{3-}, and $NH_4^+ + PO_4^{3-}$ from Dodds et al. 1989, figure 1.)

$NaHCO_3$ (4.07 kBq·mL^{-1}) was added to all samples which were incubated from 10:00 to 14:00 in situ. Incubations were terminated by filtration onto Whatman GF/F filters and samples were biochemically fractionated (Li et al. 1980) as outlined by Priscu and Priscu (1984b). Incorporated ^{14}C was apportioned into methanol-water soluble (metabolites of low molecular weight), chloroform soluble (lipid), hot-trichloroacetic acid (TCA) soluble (polysaccharide) and hot-TCA insoluble (protein) fractions.

Results

Biochemical Composition of Suspended Particulate Matter

Ratios of C:N:P in particulate matter did not indicate exclusive N or P deficiency in Flathead Lake. The molar PC:PP ratio was always above 106:1 (the ratio which has been observed for cells in balanced growth under nutrient sufficient conditions [Redfield 1958]), indicating P deficiency. The molar ratio of PC:PN was also greater than the Redfield ratio of 6.6:1, indicating N deficiency. PC:PP, PC:PN, and PN:PP ratios were the lowest during February which signifies a lower degree of nutrient limitation at this time.

We estimated non-algal PC, PN, and PP in the epilimnion during stratification (June, July, and August data) to be 61, 71, and 55%, respectively, of the total particulate content, using the linear regression method of Priscu and Goldman (1983). The mean corrected, algal C:N:P ratio during stratification was 318:16:1, suggesting a balance between N and P deficiency.

Long-Term Growth-Based Bioassays

Simultaneous additions of N and P were required to significantly increase photosynthesis in aliquots of Flathead Lake water during most of the year. In a representative time-course from June, there was no effect 4.5 d after NH_4^+ or PO_4^{3-} addition alone, but simultaneous addition of NH_4^+ and PO_4^{3-} caused about a fivefold increase in photosynthesis (Fig. 2A). The first measurable response to $NH_4^+ + PO_4^{3-}$ enrichment was observed 2.5 d after nutrient enrichment. These time-course results are similar to those obtained from experiments conducted at other times of the year. Consequently, only responses from 4.5 d are provided for the remaining experiments. A time-course of chl *a* concentration from the June experiment (Fig. 2B) showed similar trends to those obtained from carbon fixation (Fig. 2A). The simultaneous increase in chl *a* and carbon fixation following N + P enrichment that we observed indicates the increase in photosynthesis was growth-based (i.e. photosynthetic capacity did not increase).

The largest increases in carbon fixation and chl *a* were in treatments receiving simultaneous additions of NH_4^+ and PO_4^{3-}, except in February (Fig. 3). At times, there was stimulation of carbon fixation or chl *a* by NH_4^+ or PO_4^{3-} alone, but the response was never equal to that observed with simultaneous NH_4^+ and PO_4^{3-} enrichment. No other treatments stimulated growth to the same degree as simultaneous NH_4^+ and PO_4^{3-} addition. When micro-nutrients were added in addition to NH_4^+ and PO_4^{3-}, there was no additional stimulation of photosynthesis or chl *a* (Fig. 3A). Treatments with NO_3^- + PO_4^{3-} also showed significant stimulation of carbon fixa-

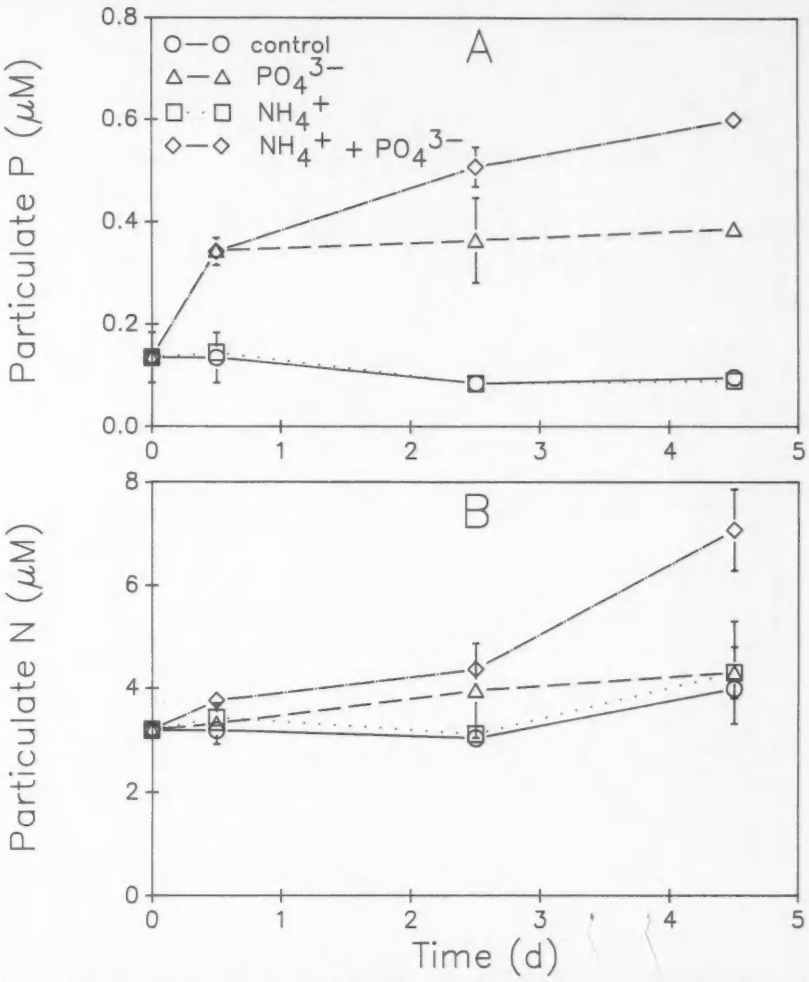

FIG. 4. Time-course of particulate P and N concentrations under different nutrient additions in Flathead Lake water for 11 July 1987.

tion and chl *a*, but they were about 45% less than increases in the $NH_4^+ + PO_4^{3-}$ treatments (Fig. 3B, 3C). Molybdenum added with $NO_3^- + PO_4^{3-}$ had no additional effect (Fig. 3B, 3C). Treatments with KCl on 14 August were not significantly different than control, indicating that KH_2PO_4 or NH_4Cl additions did not alter photosynthesis because K^+ or Cl^- was added (Fig. 3B).

In June, PP concentrations increased significantly within 0.5 d after PO_4^{3-} addition; further increases in PP were only obtained with simultaneous $NH_4^+ + PO_4^{3-}$ enrichment (Fig. 4A). Other time-courses throughout the year exhibited similar trends. Consequently, only data from 0.5 d and 4.5 d are presented for the remaining experiments.

When PP continued to increase after 0.5 d, a growth response was indicated. Particulate P had increased significantly at 0.5 d in every PO_4^{3-} enrichment except February (Fig. 5A). Simultaneous N and P deficiency was implied when only $NH_4^+ + PO_4^{3-}$ increased PP from 0.5 to 4.5 d (in June, August, and November). The increase of PP from 0.5 to 4.5 d in the presence of PO_4^{3-} alone indicated P deficiency during May (Fig. 5B).

There was never a response of particulate N (PN) to single additions of NH_4^+ or PO_4^{3-} after 0.5 d (Fig. 4B), in contrast to the rapid PP response to nutrient fertilization. Because this time-course is similar to others throughout the year, data are presented only for the 4.5 d time-point in remaining experiments (Fig. 6). There was a significant increase in PN concentration when NH_4^+ and PO_4^{3-} were added simultaneously in June, August, and November (Fig. 4B, 6).

Short-Term Bioassays

NH_4^+ enrichment significantly stimulated V_{max} for PO_4^{3-} uptake only in July (Table 2). In contrast, PO_4^{3-} adddition stimulated V_{max} for $^{15}NH_4^+$, four of six times (Table 2). Prior exposure to NH_4^+ decreased NH_4^+ uptake significantly in August only ($P<0.05$, Table 3), and rates of NH_4^+ uptake were significantly higher after 12 h prior exposure to NH_4^+ in

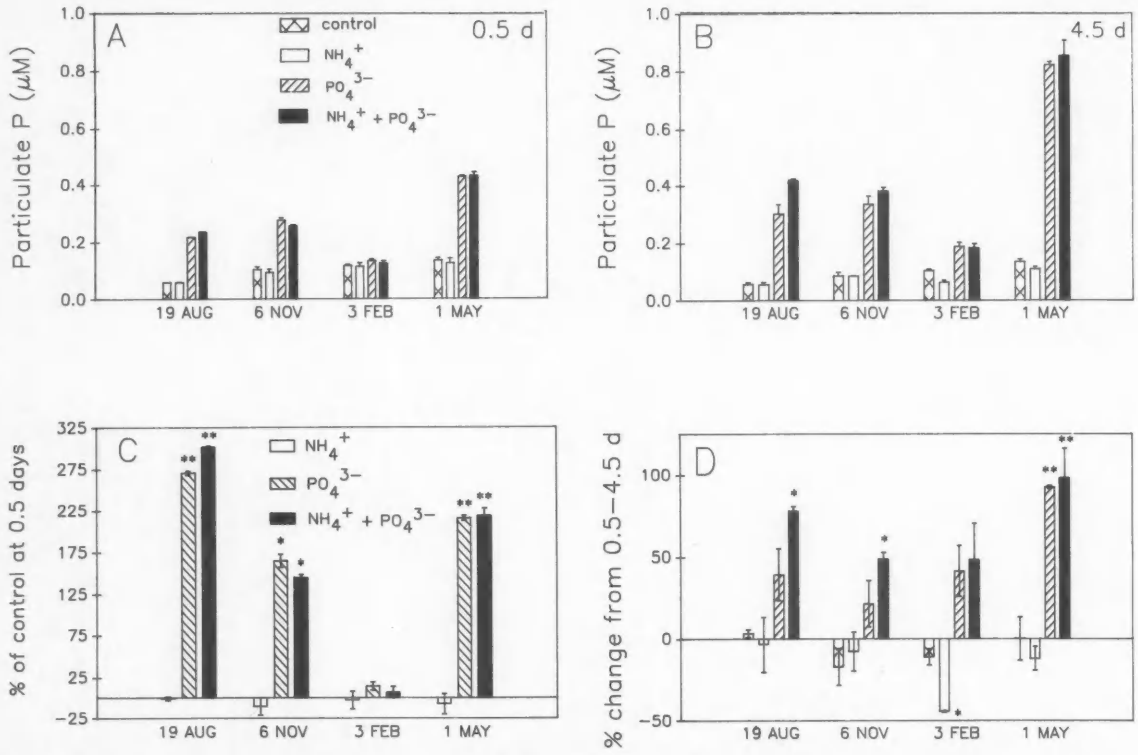

FIG. 5. Change in particulate P concentrations in nutrient enrichment experiments 0.5 d after enrichment and between 0.5 and 4.5 d after enrichment. $n = 3$ for all experiments except August when $n = 2$. Significance in C and D is determined with a Student's t-test. * = $P < 0.05$, ** = $P < 0.01$. NH_4^+ additions were 10 μM NH_4Cl and PO_4^{3-} additions were 2 μM KH_2PO_4. (A) PP concentration at 0.5 d, (B) PP concentration at 4.5 d (legend in A), (C) percent difference from control 0.5 d after fertilization, (D) percent change in particulate P concentrations between 0.5 and 4.5 d after fertilization (legend in A).

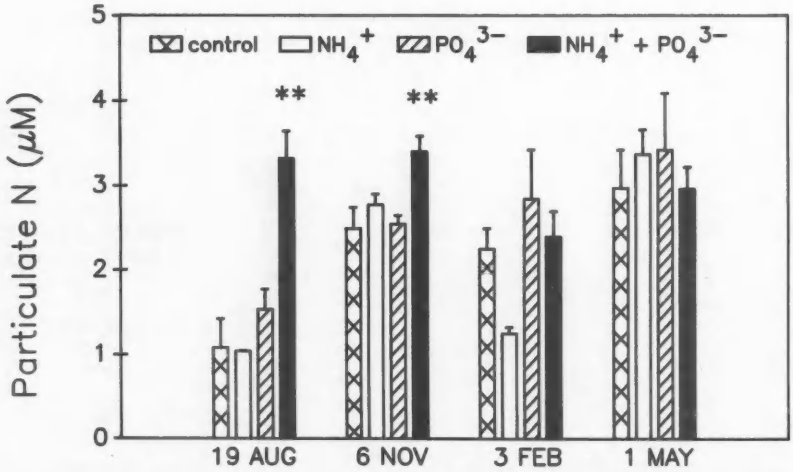

FIG. 6. Particulate N concentrations 4.5 d after nutrient enrichment in Flathead Lake. In August, $n = 2$, for the rest of the experiments, $n = 3$. Significance determined by a t-test as compared to control values, * = $P < 0.05$, ** = $P < 0.01$. NH_4^+ additions were 10 μM NH_4Cl, PO_4^{3-} additions were 2 μM KH_2PO_4.

TABLE 2. Potential nitrogen or phosphorus deficiency of phytoplankton as determined by stimulation of V_{max} of PO_4^{3-} by NH_4^+ or V_{max} of NH_4^+ by PO_4^{3-} in Flathead Lake. The $+PO_4^{3-}$ or $+NH_4^+$ treatments indicate 12 h prior exposure to NH_4^+ or PO_4^{3-}, the control treatments had NH_4^+ or PO_4^{3-} added with no pre-incubation. *$P<0.05$, t-test. $n=4$ for PO_4^{3-} uptake rates, $n=3$ for NH_4^+ uptake rates. Standard deviations are reported in parentheses.

Date	P uptake (h^{-1})	N deficiency	N uptake (h^{-1})	P deficiency
11 July 1987				
$+NH_4^+$ or PO_4^{3-}	0.18(0.01)*		0.047(0.014)*	
control	0.02(0.07)	yes	0.040(0.003)	yes
6 August 1987				
$+NH_4^+$ or PO_4^{3-}	2.21(0.23)		0.060(0.004)*	
control	3.26(1.69)	no	0.031(0.004)	yes
21 September 1987				
$+NH_4^+$ or PO_4^{3-}	1.48(0.11)		0.290(0.088)*	
control	0.64(0.70)	no	0.080(0.004)	yes
3 November 1987				
$+NH_4^+$ or PO_4^{3-}	0.85(1.06)		0.022(0.001)	
control	1.36(0.56)	no	0.022(0.001)	no
9 February 1988				
$+NH_4^+$ or PO_4^{3-}	0.06(0.03)		0.015(0.002)*	
control	0.24(0.02)	no	0.011(0.001)	yes
6 May 1988				
$+NH_4^+$ or PO_4^{3-}	0.09(0.06)		0.013(0.001)	
control	0.13(0.29)	no	0.014(0.001)	no

TABLE 3. Summary of 6 mo (June 1987–May 1988) of bioassay results from Flathead Lake. N = nitrogen deficiency, P = phosphorus deficiency, B = both nitrogen and phosphorus deficiency, 0 = no significant results ($P>0.05$). *Indicates long-term growth based bioassay, ND = not determined.

Assay	June	July	Aug.	Nov.	Feb.	May
4.5 d $^{14}CO_2$*	B	ND	B	B	O	B
4.5 d chl *a**	B	ND	B	B	O	B
4.5 d PN*	B	ND	B	B	O	O
0.5–4.5 d PP*	B	ND	B	B	O	P
0.5 d PP	P	ND	P	P	O	P
Ambient C:P	P	P	P	P	P	P
Ambient C:N	N	N	N	N	N	N
Ambient N:P	P	P	P	P	O	P
V_{max} PO_4^{3-}	N	O	O	O	O	O
V_{max} NH_4^+	P	P	P	O	P	O
NH_4^+ internal pools	N	O	O	O	O	O
PO_4^{3-} internal pools	O	O	O	O	P	O
Dark $^{14}CO_2$ response	N	O	O	N	O	O
Photosynthetic end products	N	P	O	O	O	P

September and May. Prior exposure to PO_4^{3-} decreased PO_4^{3-} uptake significantly only in February ($P<0.05$), the one time of year when the long-term bioassays indicated no P deficiency (Table 3). NH_4^+ stimulated dark carbon fixation significantly ($P<0.05$) in June and October only.

Photosynthate partitioning into protein was only stimulated by NH_4^+ during June (Fig. 7). PO_4^{3-} addition significantly decreased ^{14}C incorporation into protein in July and May, and decreased incorporation by 5% in August (Fig. 7). The data are presented as a percentage of total uptake to facilitate plotting of all data on one graph. Between-treatment comparisons of absolute $^{14}CO_2$ incorporated into protein resulted in trends identical to those observed when results were expressed in percentages.

Discussion

Seasonal Patterns of Nutrient Deficiency

Nutrient deficiency was least important during February. Light may be more limiting to growth than nutrient supply during this period. PC:PN:PP ratios deviated the least from the Redfield ratio during the winter (Fig. 1), and the growth response to nutrient enrichment was minimal during February (Fig. 3). Uptake ratios of C:N:P were closest to the Redfield ratio during February (Dodds and Priscu 1989). Total incoming irradiance is lowest during winter, and the increased depth of mixing during this period lowers irradiance experienced by the whole phytoplankton community.

Concurrent N and P Deficiencies

The phytoplankton in Flathead Lake exhibited deficiency in both N and P during most of the year. The fact that the phytoplankton community of Flathead Lake exhibited contemporaneous N and P deficiency may be related to a general trend of dual nutrient deficiency in oligotrophic systems (Dodds et al. 1989). This contrasts with the common a priori assumption that phytoplankton in freshwaters are P deficient based on statements that reduction in P loading is usually the best method to control eutrophication (e.g. Schindler 1977).

Theory and empirical data indicate that more than one nutrient can simultaneously control the primary production of a phytoplankton community (Dodds et al. 1989; Hecky and Kilham 1988). Simultaneous deficiency in P and N has been documented for many oligotrophic lakes (Lane and Goldman 1984; Morris and Lewis 1988; Suttle and Harrison 1988; Viner 1973; White et al. 1985). Some Canadian shield lakes exhibit characteristics of mild N deficiency at the same time that they exhibit P deficiency (Healey and Hendzel 1980). Other lakes also show temporal transitions between N and P deficiency (Elser et al. 1988; Kalff 1971; Prepas and Trimbee 1988; Sommer 1989; Zevenboom et al. 1982).

Comparison of Bioassays

Of the assays used in our study to assess nutrient deficiency, the strongest conclusions can be drawn from time-course measurements of growth-based changes in $^{14}CO_2$ uptake, chl *a*, PN, and PP concentrations. These long-term assays indicated simultaneous N and P deficiency except in February, when simultaneous N and P additions did not have consistent effects on algal growth. In contrast, no clear trends of N or P deficiency were apparent with the short-term bioassays.

Particulate C:N:P ratios supported the long-term bioassays because they imply both N and P deficiency in Flathead Lake except during February, especially when corrected for bacterial and detrital C, N, and P. There is a considerable precedent for using deviations of C:N:P ratios in particulate matter from Redfield ratios to indicate nutrient deficiency (see Healey 1975; Healey and Hendzel 1980; Hecky and Kilham 1988; Suttle and Harrison 1988; White et al. 1985). In Flathead Lake, PC:PP ratios indicated P deficiency year round, PC:PN ratios indicated N deficiency over the year, and N:P indicated neither when corrected for non-algal PN and PP. Long-term bioassays indicated simultaneous N and P deficiency except in February, when no clear nutrient deficiency was determined.

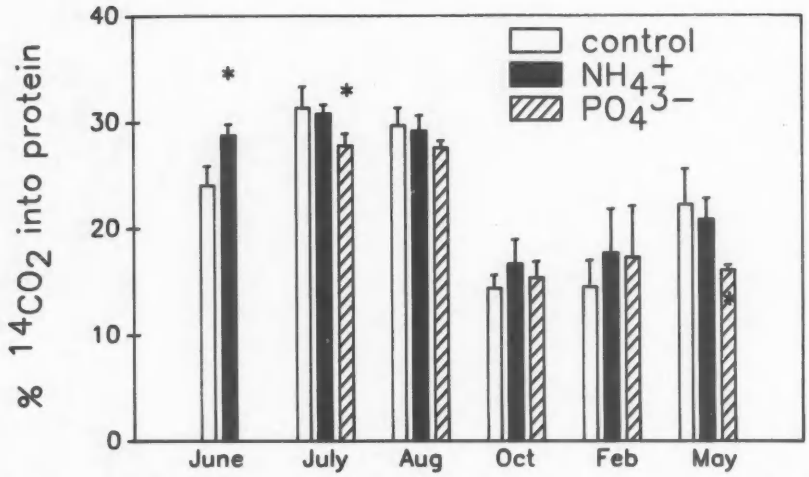

FIG. 7. Percentage of total photosynthetic $^{14}CO_2$ uptake into protein for control, and NH_4^+ or PO_4^{3-} enrichment treatments. Significance is determined with a *t*-test compared to control. * = $P<0.05$. Open bars represent unamended lake water, dark bars represent treatments with 10 μM NH_4^+ and bars with diagonal cross hatching represent treatments with 2 μM PO_4^{3-} addition.

Problems with Long-Term Bioassays

The long-term experiments may be more susceptible to container effects than experiments conducted over several hours. Several types of evidence suggest that container effects were not a problem in our 4.5 d experiments. We did not observe growth on the carboy walls during the course of our experiments and obtained similar chl *a* and photosynthetic responses to N and P enrichment in 1570 L mesocosms during July 1987 (Dodds and Priscu 1990). Although the relative abundance of phytoplankton species in the carboys changed with nutrient enrichment, the species present in the carboys were the same as in the lake phytoplankton (C. Spencer, Flathead Lake Biological Station, Polson, MT, pers. comm.). Sealing containers can also inhibit diffusion of N_2 gas, but rates of N_2 fixation in Flathead Lake were insufficient to deplete dissolved N_2 over 4.5 d (Dodds and Priscu 1990). Therefore, we conclude that container effects were negligible in our 4.5 d carboy experiments, but urge investigators using similar techniques in other systems to be aware of such effects.

Problems with Bioassays not Based on Phytoplankton Growth

Two types of problems occurred with short-term bioassays and biochemical composition of suspended particulate matter in our study: (1) short-term response did not necessarily reflect the effect of nutrient enrichment on the entire community over longer time periods (i.e. only part of the community responds to physiological bioassays), and (2) assays which focused on deficiency of only one nutrient could not indicate simultaneous deficiency.

We saw a large increase in PP concentrations in the carboy experiments after 0.5 d, except in February, which may indicate P deficiency. This assay does not rule out contemporaneous N deficiency. Such rapid luxury consumption of PO_4^{3-} can change PP concentrations and has been used as an indicator of P deficiency in a number of New Zealand lakes (Vincent 1981a). Rapid consumption of PO_4^{3-} may erroneously lead investigators to assume P limitation (Lean et al. 1990; Nyholm and Lyngby 1988).

Results from experiments designed to measure depletion of internal nutrient pools and those to measure the effect of NH_4^+ on PO_4^{3-} uptake and of PO_4^{3-} on NH_4^+ uptake only occasionally showed differences from controls. Considering the time and effort involved in these ^{32}P and ^{15}N experiments, and the fact that they only occasionally agreed with the growth-based bioassays, we do not advise using such experiments to determine nutrient deficiency in oligotrophic systems.

We obtained mixed results using NH_4^+ stimulation of dark carbon fixation as an indicator of N deficiency. It has been suggested that NH_4^+ addition should cause at least a 100% stimulation of dark carbon fixation before it conclusively shows N deficiency (White et al. 1985), and that NH_4^+ stimulation of dark carbon fixation is not a reliable indicator of combined demand for inorganic N by heterocystous cyanobacteria (Vincent 1981b). Furthermore, carbon fixation of NH_4^+ oxidizing bacteria can be stimulated by NH_4^+, which may confound results (W. K. Dodds and J. C. Priscu, unpubl. data).

The PO_4^{3-} depression of protein synthesis results are provocative and suggest that this bioassay may provide a technique for short-term indication of PO_4^{3-} deficiency. However, this assay requires more study. It has been documented that the apportionment of $^{14}CO_2$ into various molecular fractions can be influenced by day length and temperature (Cuhel and Lean 1987). The factors which control the rates of protein synthesis in phytoplankton are not well enough understood for us to recommend using PO_4^{3-} depression of photosynthesis as a reliable bioassay.

Summary

Several-day enrichment bioassays based on growth responses may allow lake managers to make more informed decisions about nutrient deficiency than would be possible on the basis of shorter-term bioassays or routine monitoring of in situ conditions. Long-term bioassays which monitor increases in bio-

mass were more consistent as indicators of nutrient deficiency than short-term measurements of NH_4^+ enhancement of carbon fixation, and effects of nutrient enhancement on NH_4^+ and PO_4^{3-} uptake rates in Flathead Lake. Results from short-term bioassays were not always consistent (Table 3), may have been confounded by bacterial metabolism, and could have reflected short-term (min-h) variations in physiological state related to factors other than nutrients (i.e. light, temperature, etc.). Short-term (h) experiments (dark $^{14}CO_2$ uptake enhancement by NH_4^+, and alkaline phosphatase activity) can correlate with longer-term nutrient enrichment experiments (St. Amand et al. 1989), so such experiments may not be without value in all systems. However, Hecky and Kilham (1988) concluded that short-term bioassays may not be reliable indicators of nutrient deficiency, and we repeatedly show this to be true in a large oligotrophic lake.

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Distribution of Potentially Harmful Phytoplankton Species in the Northern Strait of Georgia, British Columbia

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Haigh, R., and F. J. R. Taylor. 1990. Distribution of potentially harmful phytoplankton species in the northern Strait of Georgia, British Columbia. Can. J. Fish. Aquat. Sci. 47: 2339–2350.

Spatial and temporal distributions of *Chaetoceros convolutum* (including *C. concavicorne*), *Heterosigma akashiwo*, *Protogonyaulax* (= *Alexandrium*) *catenella*, and *Dinophysis* are presented for the northern Strait of Georgia from March to September, 1986. In spring *C. convolutum* was found on the slightly stratified eastern side in concentrations just below those leading to kills of caged salmon (4-5000 cells·L^{-1}). The northern Strait became thermally stratified in summer. At this time the population centre of *C. convolutum* shifted to mid-Strait near the edge of the Discovery Passage tidal jet where it was maintained near the pycnocline (10–15 m) in concentrations up to 35,000 cells·L^{-1}. *Heterosigma akashiwo*, another fish killer, was the photosynthetic dominant in June and reached 200 000 cells·L^{-1} in the northwest at 5 m. *Protogonyaulax catenella*, a source of paralytic shellfish poison (PSP), was also most abundant in the north and west, where dangerous levels of PSP often occur, particularly near Comox. Collectively, *Dinophysis* species exceeded the critical concentration for diarrheic shellfish poisoning (200 cells·L^{-}) on the western side during summer. All the potentially harmful flagellated species seem to be introduced from the northern passageways. Sheltered bays to the north are possible seed beds for cyst-forming species whereas *C. convolutum* remains in the Strait year-round.

Des distributions spatio-temporelles de *Chaetoceros convolutum* (y compris *C. concavicorne*), *Heterosigma akashiwo*, *Protogonyaulax* (= *Alexandrium*) *catenella*, et *Dinophysis* sont présentées pour le nord du détroit de Géorgie, de mars à septembre 1986. *Chaetsceros convolutum* a été trouvé au printemps sur le côté est légèrement stratifié, en concentrations juste inférieures à celles qui produisent la destruction des saumons en cage (4 à 5 000 cellules·L^{-1}). La partie nord du détroit comporte des strates thermiques en été. À ce moment-là, le centre de la population de *C. convolutum* se déplace vers le milieu du détroit à proximité du bord du courant-jet tidal du passage Discovery où elle est maintenue près de la pycnocline (10–15 m) en concentrations pouvant atteindre 35 000 cellules·L^{-1}. *Heterosigma akashiwo*, une autre espèce destructrice de poisson, représentait l'espèce photosynthétique dominante en juin et atteignait 200 000 cellules·L^{-1} dans le nord-ouest à 5 m. *Protogonyaulax catenella*, source de l'intoxication paralysante par les mollusques, était aussi assez abondante dans le nord et dans l'ouest, où l'on enregistre souvent des teneurs dangereuses en toxine responsable de l'intoxication paralysante, notamment près de Comox. Collectivement, les espèces de *Dinophysis* dépassaient la concentration critique de la toxine responsable de l'intoxication diarrhéique par les mollusques (200 cellules·L^{-1}) sur le côté ouest, pendant l'été. Toutes les espèces de flagellés qui peuvent être dommageables semblent provenir des voies situées au nord. Des baies protégées au nord constituent des lits de semence possibles pour les espèces formatrices de kystes tandis que *C. convolutum* demeure dans le détroit toute l'année.

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The aquaculture industry in British Columbia has become increasingly aware of its susceptibility to certain species of marine phytoplankton. Fish kills occur as a result of blooms of harmful species which can either deplete the water's oxygen supply, introduce toxins, or physically damage the gills (Gaines and Taylor 1986). On the other hand, shellfish growers must cope with the bio-accumulation of paralytic, diarrheic, and other neurotoxic poisons due to the ingestion of toxic phytoplankton.

The northern Strait of Georgia (hereinafter designated NSG) is of special interest due to considerable aquaculture activity along its shores as well as the passages through islands to the north. The NSG may act as a source for certain harmful species, transfer populations from one region to another, or act as an effective barrier to dispersal. Our objective is to pinpoint areas in the NSG which are most susceptible to harmful algal blooms. Data concerning the microplankton in general are presented in Haigh (1988b) and Haigh and Taylor (1991). Only those species which are known to be harmful are presented here.

Of immediate concern to the finfish industry are the diatoms *Chaetoceros convolutum* and *C. concavicorne* which possess long hollow setae armed with secondary spines. In general, the setae of *C. concavicorne* flare out from their bases whereas those of *C. convolutum* retain a constant diameter. The spines are usually more developed in the former. They frequently co-occur and due to occasional uncertainty in separating these two species, they have been grouped into *C. convolutum* for this paper. Bell (1961) found that the spines of *C. convolutum* penetrated the gill tissue of ling cod and suggested death might result due to capillary haemorrhage, anoxia by stimulation of heavy mucus secretion, or microbial invasion of damaged tis-

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sue. Bell et al. (1974) determined that the filtrate of *C. convolutum* was not toxic. Concentrations of as few as 1000 cells·L^{-1} have been claimed to be harmful to salmon, especially sockeye, due to tearing of delicate gill membranes (Kennedy et al. 1976). Juvenile chum salmon exposed to high concentrations of *C. convolutum* (≈10^5 chains·L^{-1}) died from gill dysfunction caused by clogging and destruction of gill epithelium (Farrington 1988); death was effectively due to hypoxia. Horner et al. (1990) reported that Atlantic salmon reared in Washington waters were far more susceptible to *C. convolutum* than Pacific salmon.

The other major species of concern to fish farmers is *Heterosigma akashiwo* (formerly identified in North America as *Olisthodiscus luteus*) which reached densities of 10^8 cells·L^{-1} in adjacent Sechelt Inlet in the summer of 1986 (Dale et al. 1987). How this raphidophyte flagellate kills fish is uncertain but it is thought to release a toxin which alters gill permeability; there is prolific mucus produced by the gills.

Most of the causes of shellfish toxicity are dinoflagellates. These species are ingested by shellfish which are unharmed by them. The toxins accumulate in the body tissues of the bivalves so that eventually humans are at risk should they consume high doses. In British Columbia the main dinoflagellate genus of concern in *Protogonyaulax*, called *Alexandrium* by recent authors (e.g. Balech 1985). *Protogonyaulax catenella* and *P. tamarensis* (the latter including *P. acatenella*, which appears to be a morphological variant of it (Cembella 1986)) can cause paralytic shellfish poisoning (Prakash and Taylor 1966; Quayle 1969; Gaines and Taylor 1986). Geographic and temporal patterns of PSP toxicity in British Columbia waters were analysed by Gaines and Taylor (1985). The spread of PSP in Puget Sound was documented by Horner et al. (1990). *Goniodoma pseudogonyaulax*, which is related to *Protogonyaulax*, has yet to be linked to PSP but may be important in British Columbia waters. Diarrheic shellfish poisoning (DSP) can result if bivalves have been consuming relatively low numbers of certain *Dinophysis* spp. (Murata et al. 1982; Kat 1983; Yasumoto et al. 1984; Lassus et al. 1985; Osaka and Takabayashi 1985; Yasumoto 1985; Cembella 1989). This report includes the collective distributions of *Dinophysis acuminata, D. fortii, D. ovum*, and *D. norvegica* over the study period.

Other species may occasionally be harmful but are usually not a problem in this region (see Gaines and Taylor 1986). *Dictyocha speculum* is a photosynthetic silicoflagellate that has been implicated in a fish kill in Danish coastal waters (Thomsen and Moestrup 1985; Jochem and Babenerd 1989). *Noctiluca scintillans* is a dinoflagellate which frequently causes "red water" blooms in British Columbia and has a large fluid-filled vacuole with very high concentrations of ammonium (Kesseler 1966). Although *N. scintillans* has been harmful in other parts of the world (Enomoto 1956), blooms of this organism in British Columbia have yet to cause damage. A culture of *Chrysochromulina polylepis*, the cause of massive marine fauna mortalities in Scandanavia in 1988 (Dahl et al. 1989; Lindahl and Dahl 1990), was isolated from the Northeast Pacific but was subsequently lost. It was not observed in this study.

Materials and Methods

For the sake of clarity this paper describes the main axis (34 km) of the NSG (Fig. 1) as running north to south and the cross-Strait axis (26 km) as being east-west. The depth of the Strait varies considerably from shallow shoals to the deep central basin (≈300 m). The physical oceanography of the Strait of Georgia was described in some detail by both Tully and Dodimead (1957) and Waldichuk (1957). LeBlond (1983) provides a more recent account. The biological oceanography of the Stait was reviewed by Harrison et al. (1983) who emphasized the need for more phytoplankton studies in the northern region.

During 1986, sampling was undertaken five times on five transects (Fig. 1). The first three cruises (March 18–19, April 22–23, and June 25–26) were aboard the *C.S.S. Vector*, the last two (August 12–13 and September 16–17) aboard the *C.S.S. Revisor*. Due to the behaviour of certain phytoplankton species, notably vertically migrating dinoflagellates, sampling was always done during the daylight hours in an effort to minimize some of the variation due to diel periodicity.

A pump sampling system hooked up to a fluorometer, was employed so that fluorometric traces of the top 20 m of the water column were obtained. Once the chlorophyll profile was known samples were taken from the surface, 20 m, and two intermediary depths. They were analysed for chlorophyll (Parsons et al. 1984), nitrate (Wood et al. 1967), and plankton (Hasle 1978). The latter analysis was performed for only three of the five stations along Transects 1, 3, 4, and 5 due to the large amount of time needed for full plankton enumeration.

To determine possible controlling factors, the species data (from all sample times for *C. convolutum* and the latter three sample times for the flagellated species, which were only present in appreciable numbers from June onward) were put through a multiple regression analysis with five independent variables: density stratification, determined as the absolute difference in sigma-*t* (σ_t) units from the surface to 20 m; pycnocline depth, determined as the depth at which a density change over 3 m was maximal; surface temperature, which was thought to be a good indicator of seasonality; surface salinity; and surface nitrate. The latter three variables, all surface-related, were used because most of the special-interest species were flagellated and most common in surface layers (Haigh 1988b). The independent variables, having different units of measurement, were standardized to avoid differential weighting. The dependent variable was always an average concentration (cells·L^{-1}), being the mean of four depths, and was transformed with natural logarithms. After an initial regression the residuals were Studentized and those values lying outside the 95% C.I. were removed. The remaining observations were then put through the regression again.

Results

Species Distributions

In March *Chaetoceros convolutum* favoured the stratified eastern side, reaching a subsurface (5 m) maximum of 3000 cells·L^{-1} near Desolation Sound (Fig. 2A). This diatom was also most numerous on the eastern side in April (Fig. 2B); however, the cells were fairly well mixed over 20 m, reaching a maximum of 4300 cells·L^{-1} in surface waters at Stn 3e. By June (Fig. 2C) the population was minimal and its centre had moved to the mid-NSG where it stayed for the remainder of the study period. Highest densities were reached in August (maximum >35 000 cells·L^{-1}, Fig. 2D). The majority of subsurface maxima occurred between 6 and 20 m, with a tendency to be progressively deeper from west to east. *Chaetoceros convolutum* numbers were reduced by September

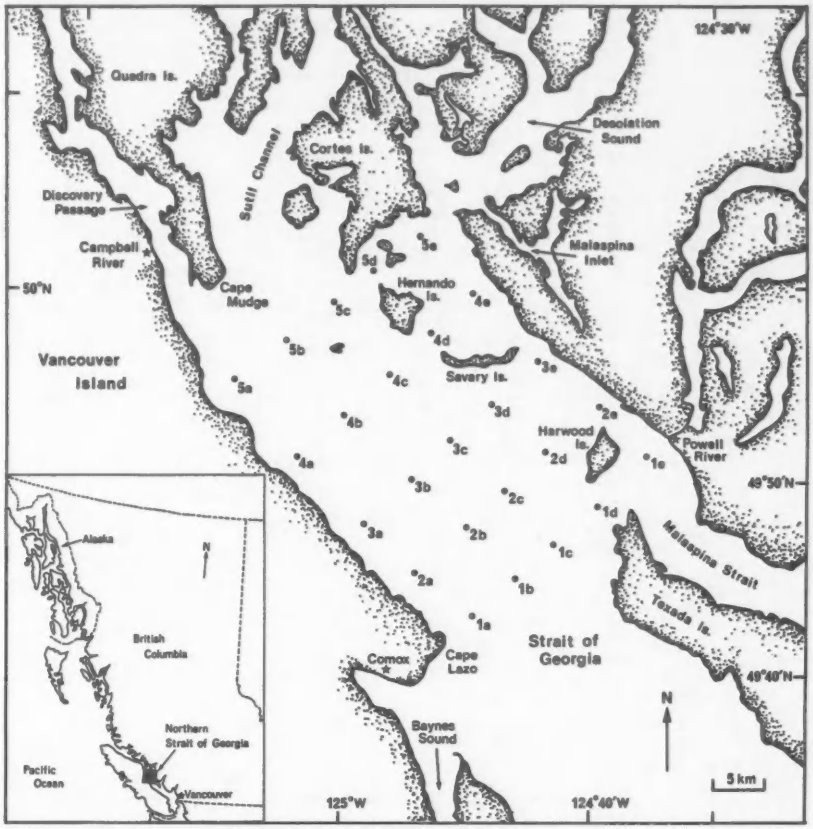

FIG. 1. Northern Strait of Georgia with station positions.

(Fig. 2E) and formed subsurface maxima between 4 and 20 m with no surface concentrations.

In June *Heterosigma akashiwo* had become the major photosynthetic dominant with a peak concentration of 200 000 cells·L^{-1} at 5 m in the northwest tidal jet (Fig. 3A). Maxima occurred in the upper 10 m on the western side and below 10 m on the east. In August (Fig. 3B) this raphidophyte was still most abundant in the tidal jet area, reaching 14 500 cells·L^{-1} in surface waters. Peak concentrations in September occurred in the surface waters of mid-Strait (54 000 cells·L^{-1}, Fig. 3C). Later in the season *H. akashiwo* preferred surface waters.

In June (Fig. 4A) *Protogonyaulax catenella* was most concentrated in surface waters of the northwest with a maximum density of 2900 cells·L^{-1} near the tidal jet. By August (Fig. 4B) maxima were subsurface with a peak concentration of 1100 cells·L^{-1} at 9 m off Cape Lazo. In September (Fig. 4C) concentrations had increased once more and were highest in the upper 5 m of the western stations (maximum >2400 cells·L^{-1}). Overall, *Protogonyaulax* appeared to prefer the western side with possible injection from the northern passageways.

The four main *Dinophysis* species observed in the NSG were combined for analysis. In June *Dinophysis* appeared to be stimulated by the tidal jet so that concentrations were highest on the western side (Fig. 5A). At most of the stations these dinoflagellates occupied the upper 10 m. A similar pattern prevailed in August (Fig. 5B) with vertically-mixed distributions in the north and concentrations in the upper 10 m in the south. By September (Fig. 5C) *Dinophysis* numbers had decreased with the largest concentration (maximum = 500 cells·L^{-1}) appearing to intrude from Sutil Channel. The Hernando–Savary basin in the northeast was the new population centre and the northwest tidal jet no longer stimulated growth.

Multiple Regression

Stratification favoured *H. akashiwo* ($P<0.05$) but negatively affected *C. convolutum* ($P<0.001$) (Table 1). Surface temperature positively affected *C. convolutum* ($P<0.001$) significantly and *Dinophysis* slightly ($P<0.10$); i.e. the concentration of these species increased with warmer temperatures and/or summer conditions. *Heterosigma akashiwo* increased with surface salinity ($P<0.05$) as did *P. catenella* ($P<0.10$), whereas *C. convolutum* decreased ($P<0.05$). Most species concentrations increased with higher nitrate levels, indicating the limiting effect on growth when surface nitrate was stripped in August and September.

The regression model works best for *C. convolutum* ($F=6.81$) and *H. akashiwo* ($F = 20.7$). Most of the explanatory variables were successful in describing these two species, pycnocline depth being the exception. Unfortunately, as in most field studies, there are problems with multicolinearity (Cooper and Weekes 1983). Stratification is inversely correlated with

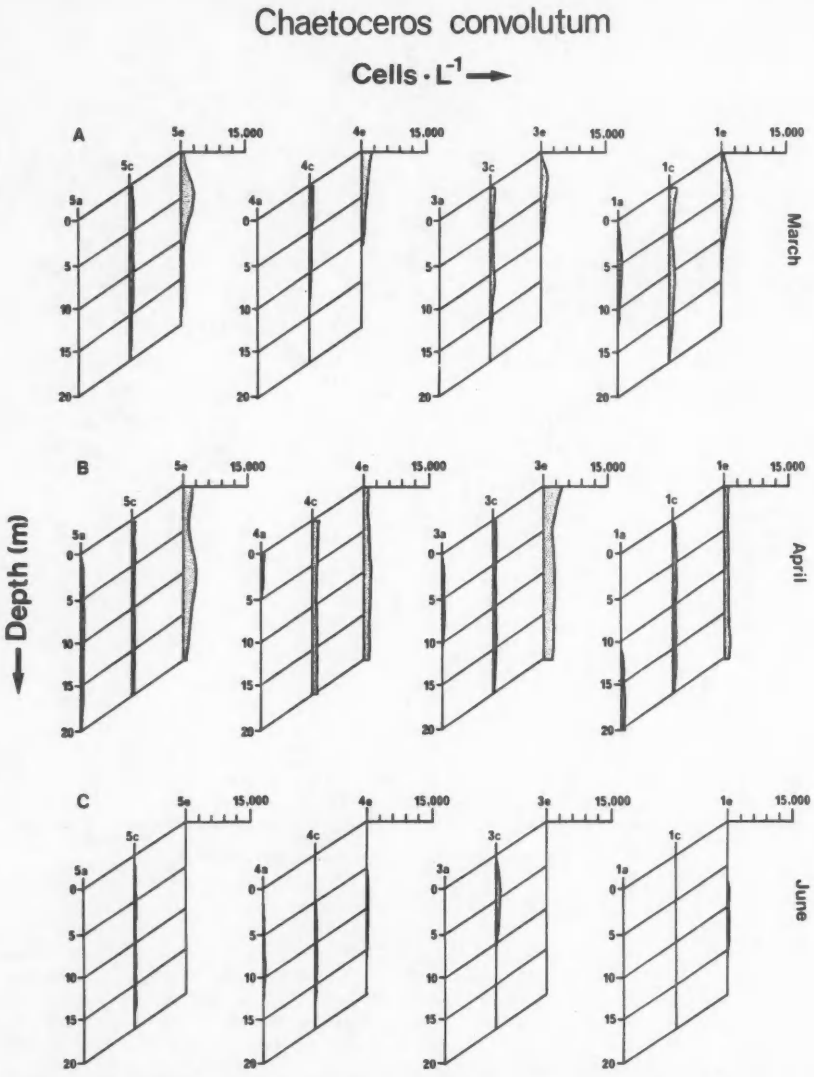

FIG. 2. Depth distributions of *Chaetoceros convolutum* along Transects 1, 3, 4, and 5 in the northern Strait of Georgia, 1986. (A) March 18–19. (B) April 22–23. (C) June 25–56. (D) August 12–13. (E) September 16–17. (*Fig. 2 concluded next page*)

salinity, an interesting fact considering that the main source of stratification over the study period was due to seasonal temperature flux. Also, temperature is inversely correlated with nitrate, reflecting the fact that as the summer progressed surface nitrates were depleted by the phytoplankton populations.

Discussion

According to Brett et al. (1978), blooms of *Chaetoceros convolutum* developed in Departure Bay from 1973 to 1976 between mid-September and mid-November, with the worst consequences for salmon occurring in October. Gaines and Taylor (1986) reported that *C. convolutum* is most abundant from September to February but under unusual weather conditions it can even bloom in summer (Sechelt Inlet, June, 1988, unpubl. observ.). In 1986 sampling was started in early spring so that winter populations were unknown. During the spring sampling period, *C. convolutum* was more favoured by the weakly stratified eastern side where Fraser River runoff alters the vertical salinity structure year-round (Tully and Dodimead 1957; Mackas et al. 1980). Concentrations also appeared to be elevated in Desolation Sound. The "*concavicorne*" form was prevalent in April.

Once thermal stratification had been established the *C. convolutum* populations became concentrated in mid-NSG, associated with the boundary of the Discovery Passage tidal jet. In June the water column was most stratified (average $\delta\sigma_t = 3.24$) and *C. convolutum* concentrations were very low, a situation

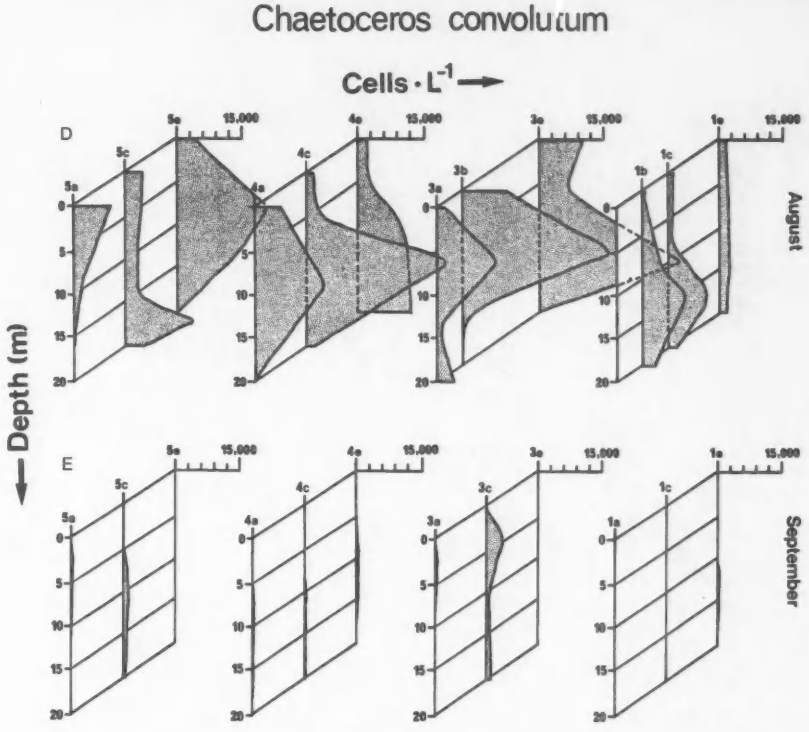

FIG. 2. (*Concluded*)

highlighted by the regression (Table 1). *Chaetoceros convolutum* numbers increased in August along with those of the other *Chaetoceros* species. The smaller "*convolutum*" form accounted for the majority of the August population, which reached 35 000 cells·L^{-1}, a level considerably in excess of the 4–5000 thought to be harmful to caged fish.

At highest concentrations, *Chaetoceros convolutum* was found near 10 m in the region of the pycnocline (Haigh 1988b) where it had possibly settled out from nitrate-depleted surface waters. It could be suggested that conditions at the pycnocline were perhaps conducive to growth, at least in areas of higher tidal turbulence. In the region of the pycnocline light and temperatures are low, and nutrients high; conditions characteristic of winter. *Chaetoceros convolutum* appears to be a low-light species. The presence of plastids within the setae, unlike most *Chaetoceros*, might be an illumination adaptation. Consequently, conditions can exist in summer such that populations of this diatom may increase to harmful proportions at depth.

During the June sampling period *Heterosigma akashiwo* was the photosynthetic dominant with respect to biomass (Haigh 1988b). This raphidophyte was abundant at all depths sampled, with higher concentrations in the upper 10 m. Yamochi (1984) found that *H. akashiwo* exhibited diel vertical migration, preferring surface waters during the day for photosynthesis. This migration is not induced by phototaxis as the cells begin migrating a few hours before a change in light phase (Hatano et al. 1983; Wada et al. 1985), presumably due to a circadian rhythm (Kohata and Watanabe 1986). Swimming velocities are in the range of 1.0–1.3 m·h^{-1} (Yamochi and Abe 1984). The downward migration is beneficial when surface nutrients are depleted because the cells can spend part of their time at the nutricline (Watanabe et al. 1988). Lipid accumulation is coupled with migration and peaks near the end of the dark cycle (Wada et al. 1987) which might aid upward motility. *Heterosigma akashiwo* was especially numerous near the Discovery Passage tidal jet. In nearby Sechelt Inlet at this time concentrations of 10^8 cells·L^{-1} were observed.

Tomas (1980) suggested that in Narragansett Bay, where a yearly bimodal pattern occurs, temperature is one of the more important factors regarding *Heterosigma*. He found that abundance peaks co-occurred with minimal deviations from 15°C, the flagellate's optimal temperature. Surface waters of the NSG averaged 13.8°C in June (Haigh 1988a), and the regression indicates a strong preference for lower temperatures (Table 1) given a summer range of 12.0–18.6°C. Japan excystment data agrees. This suggests that blooms may be seeded from shallower areas and advected into the central NSG by currents. Our time resolution did not permit us to observe the direction(s) of spread.

Stratification was also a good predictor for *H. akashiwo* in the regression model, due chiefly to June's greater stratification values. Surface nitrate was replete in June so that these selecting forces coupled with *Heterosigma*'s known inhibitory effects on other planktonic organisms (Pratt 1966; Honjo et al. 1978; Tomas 1980; Egloff 1986; Verity 1987; Taniguchi and Takeda 1988) perhaps afforded it dominance. Once surface nitrate depletion had occurred (August), *H. akashiwo* densities were greatest in areas of higher tidal streaming: near the northwestern jet and between Savary Island and Malaspina Peninsula. Concentrations, however, were markedly lower than in June. By

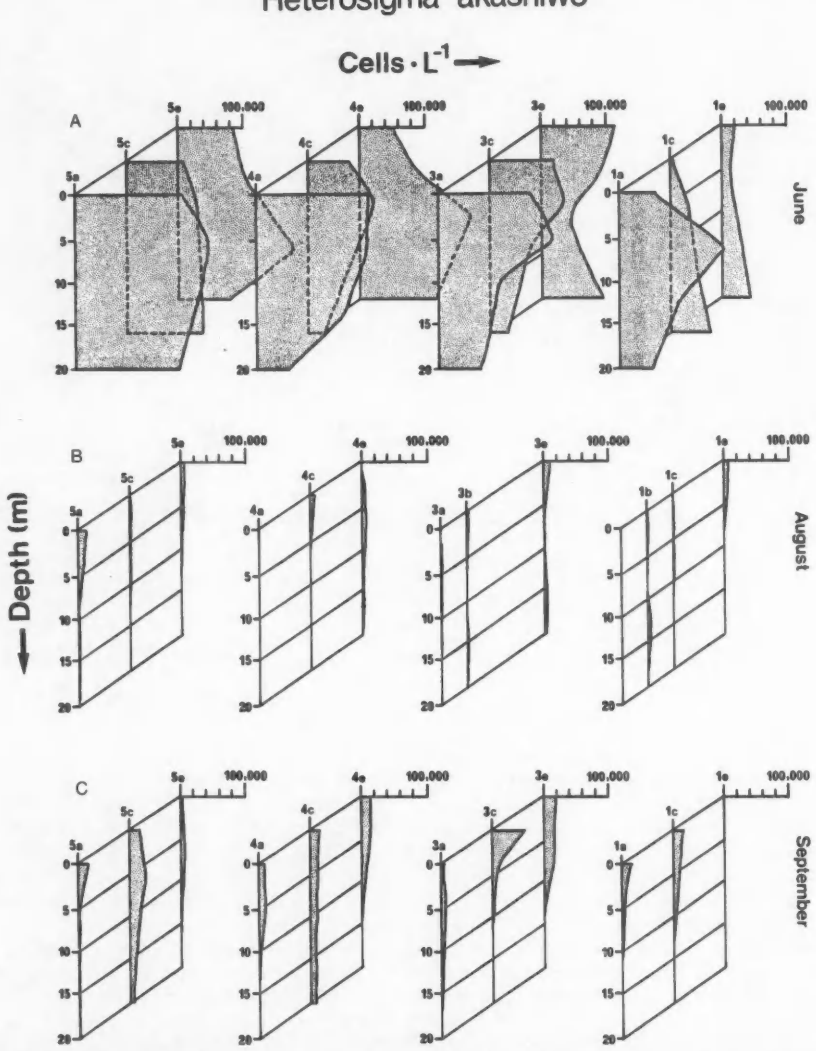

FIG. 3. Depth distributions of *Heterosigma akashiwo* along Transects 1, 3, 4, and 5 in the northern Strait of Georgia, 1986. (A) June 25–26. (B) August 12–13. (C) September 16–17.

September, *H. akashiwo* abundance had increased but densities were still low. At this time the population centre had shifted to mid-NSG, remaining in the path of the tidal jet.

Yamochi (1983) concluded that in Osaka Bay, Japan, chelated iron from river runoff during the rainy season was a critical factor controlling *H. akashiwo* outbreaks. Nishijima and Hata (1984) found that vitamin B_{12} was the only factor essential for growth and suggested that *H. akashiwo* was ultimately limited by B_{12} concentrations. Cattell (1969) found that this vitamin was more abundant after the spring bloom in the Strait of Georgia, and attributed this observation to generation from decaying plankton by bacteria. This could be one reason that *Heterosigma* red tides most often follow the spring bloom. Generally, the NSG B_{12} levels are lower than those in the southern Strait (Cattell 1969). In 1986 there appeared to be an absence of the spring bloom in the NSG (Haigh and Taylor 1991) which might lead to lower B_{12} values. However, the Fraser River influence is present along the eastern side (Tully and Dodimead 1957; Mackas et al. 1980), a situation which should elevate B_{12} levels. Also, vitamin B_{12} concentrations in shallower adjacent waters would be greater so that *Heterosigma* growth would not be limited, and blooms of this flagellate could be advected into the NSG.

The two local shellfish poison-producing species of *Protogonyaulax*, *P. catenella*, and *P. tamarensis* are usually geographically separated in British Columbia coastal waters (Taylor 1984). The former occurs chiefly on the open coast as well as in Juan de Fuca Strait and Puget Sound, whereas the latter appears confined to more estuarine areas of the eastern Strait of Georgia. It is questionable whether these two *Protogonyau-*

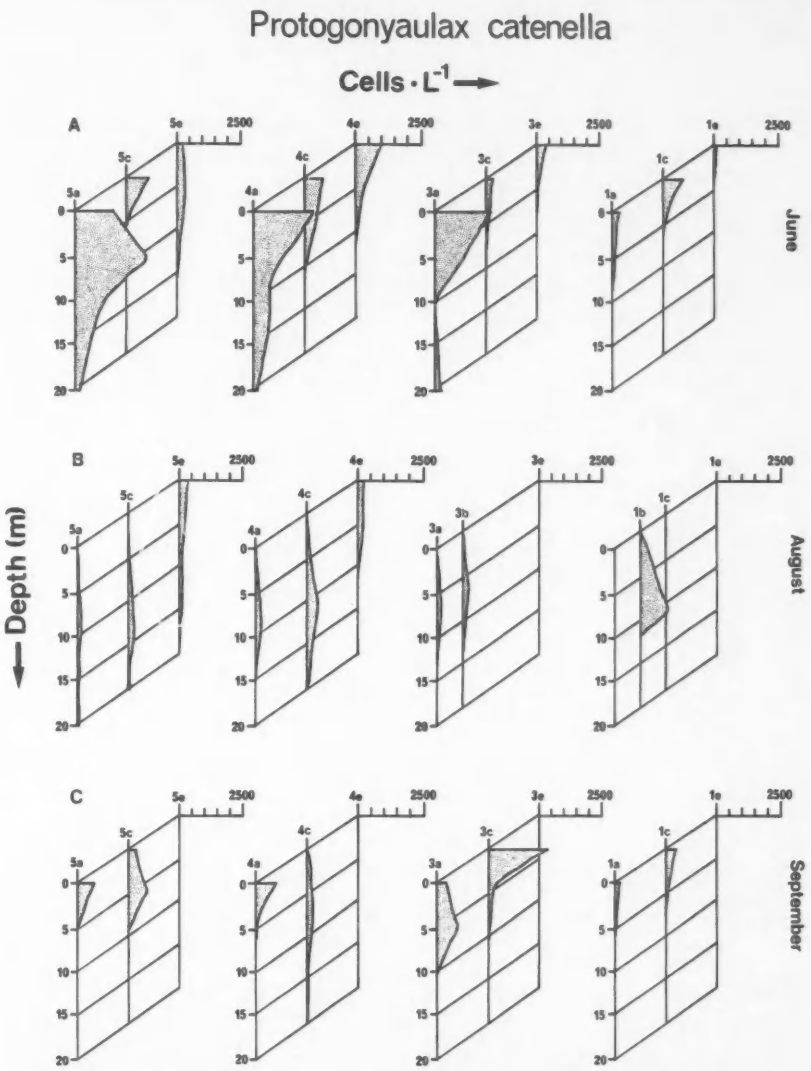

FIG. 4. Depth distributions of *Protogonyaulax catenella* along Transects 1, 3, 4, and 5 in the northern Strait of Georgia, 1986. (A) June 25–26. (B) August 12–13. (C) September 16–17.

lax should be separated at the species level (Cembella and Taylor 1985, 1986; Cembella 1986; Cembella et al. 1987, 1988).

Data for the western NSG have been sparse until now. This study shows that *Protogonyaulax catenella* is found throughout the NSG (as well as the Malaspina Inlet complex) but seemed to prefer the northern regions and the western side. From June to September there was a shift southward of the population maximum. Thus it appears that the occurrence of PSP on the east coast of Vancouver Island in the Campbell River/Comox region is due to the southward spread of *P. catenella*.

Paralytic shellfish poisoning can occur even if *Protogonyaulax* is present in low numbers because shellfish can filter tens of litres of seawater per day (Gaines and Taylor 1986). Sakshaug and Jensen (1971) stated that concentrations of approximately 4000 cells·L^{-1} of *P. tamarensis* would maintain a constant toxic level in mussels, assuming the lowest rate of toxin depuration and the highest toxicity reported for dinoflagellates. Gaines and Taylor (1986) reported that a lethal dose could be accumulated if *Protogonyaulax* concentration is 10 000 cells·L^{-1}. The maximum concentration attained in this study was 2900 cells·L^{-1} during June.

Shellfish are not the only planktivores intoxicated by these dinoflagellates. White (1981) and Hayashi et al. (1982) analysed the toxin content of selected zooplankters which had been feeding on *P. tamarensis* (= *G. excavata*) and found that toxins were rapidly accumulated. Further bioaccumulation along the food chain can occur and kills vertebrates: sea birds via small fish and/or crustaceans (McKernan and Scheffer 1942); sand eels via the cladoceran *Evadne nordmanni* (Adams et al. 1968), shags via sand eels (Coulson et al. 1968); herring via the pteropod *Limacina retroversa* (White 1977); herring via *E. nord-*

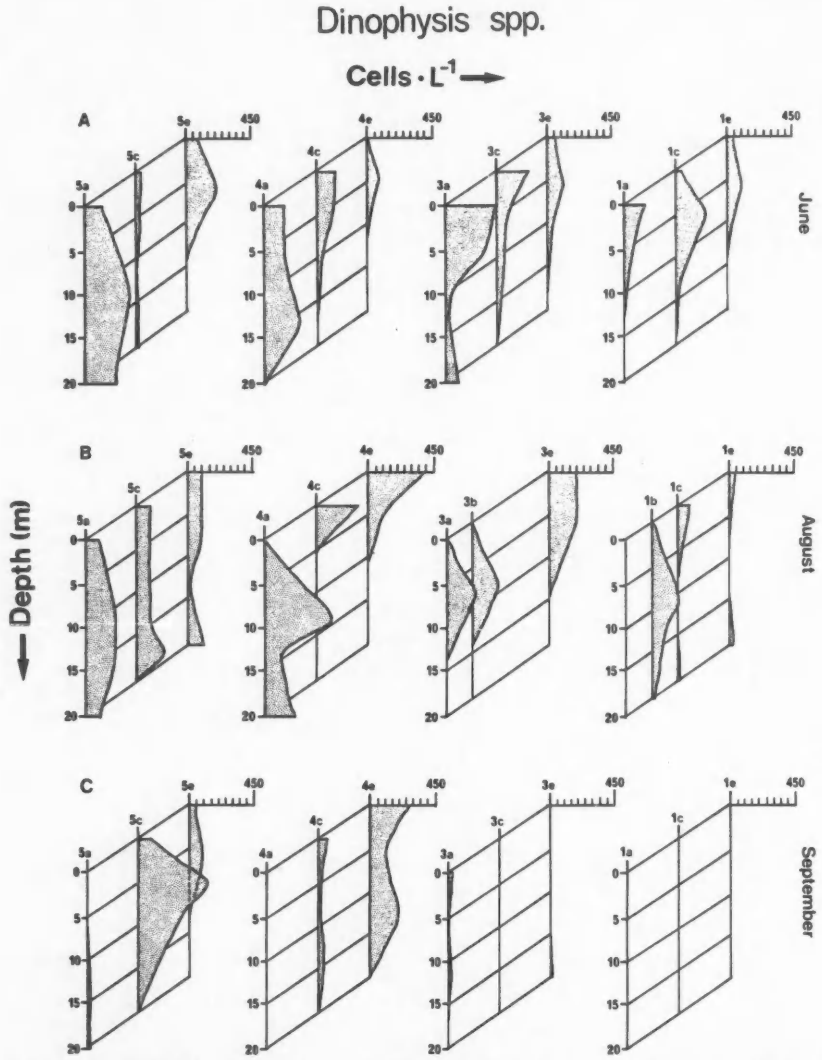

FIG. 5. Depth distributions of *Dinophysis* spp. along Transects 1, 3, 4, and 5 in the northern Strait of Georgia, 1986. (A) June 25–26. (B) August 12–13. (C) September 16–17.

manni (White 1980); and humpback whales via Atlantic mackerel (Geraci et al. 1989). Direct fish kills at the Faroe Islands, including those in farms, apparently resulted from a very dense *P. tamarensis* (= *G. excavata*) bloom (Mortensen 1985).

Goniodoma pseudogonyaulax is closely related to *Protogonyaulax* but has not been shown to produce toxin. A. D. Cembella (Maurice Lamontagne Inst., P.O. Box 1000, Mont-Joli, Que., G5H 3Z4, Canada, pers. comm.) recently found no toxicity from natural samples using HPLC and ELISA techniques. This species has been studied in brackish water lagoons in France (Biecheler 1952), Obidos Lagoon in Portugal (Silva 1965), and Japanese rockpools (Kita et al. 1985; Kita and Fukuyo 1988). We see *G. pseudogonyaulax* in our coastal waters during the summer and fall. It prefers the northern and western waters of the NSG and is most abundant during August and September. There appears to be intrusion of this dinoflagellate from Sutil Channel in September.

Certain dinophysoid dinoflagellates have been circumstantially linked to toxins responsible for diarrheic shellfish poisoning (DSP) leading to diarrhea, nausea, vomiting, abdominal pains, and headache (Yasumoto et al. 1980; Yasumoto et al. 1984; Krogh et al. 1985; Underdal et al. 1985). No *Dinophysis* species has ever been cultured; therefore, direct evidence for toxin production is not available. Although official records of this phenomenon are lacking in British Columbia, we suspect that DSP has probably been misdiagnosed many times in the past.

Dinophysis acuminata appears to be responsible for DSP in European coastal waters. The French monitor this dinoflagel-

TABLE 1. Species regressions. Independent variables: Density stratification ($\delta\sigma_t$ from 0 to 20 m), pycnocline depth (m), surface temperature (°C), surface salinity (ppt), surface nitrate (μM). *** $P<0.001$, ** $P<0.05$, * $P<0.10$.

Independent variables	Regression	*Chaetoceros convolutum*	*Heterosigma akashiwo*	*Alexandrium catenella*	*Dinophysis spp.*
Intercept	Coeff	5.92	9.14	3.98	3.85
	t-val	28.2***	58.4***	10.9***	19.3***
Stratification	Coeff	−3.45	1.17	0.983	−0.149
	t-val	−3.87***	2.98**	1.05	−0.380
Pycnocline depth	Coeff	0.411	0.234	0.335	−0.205
	t-val	1.67	1.43	0.863	−0.986
Surface temperature	Coeff	2.72	−1.04	−0.420	0.582
	t-val	5.04***	−4.49***	−0.761	1.98*
Surface salinity	Coeff	−2.44	0.734	1.46	−0.419
	t-val	−3.03**	2.08**	1.72*	−0.930
Surface nitrate	Coeff	1.67	0.669	0.307	0.612
	t-val	3.90***	2.90**	0.554	2.05**
Statistics	*F*-val	6.81***	20.7***	1.67	2.47*
	R^2	0.400	0.787	0.218	0.307
	df	5,51	5,28	5,30	5,28

late over summer and close shellfish marketing when concentrations exceed 200 cells·L^{-1} (Lassus et al. 1985). Kat (1983), however, found that only those blooms of greater than 20 000 cells·L^{-1} were followed by DSP in the Dutch Waddensea. In the NSG *D. acuminata* exceeded 200 cells·L^{-1} during the June and August samplings.

Dinophysis fortii causes DSP in northern Japan from spring to summer when water temperatures are 10–18°C (Osaka and Takabayashi 1985). If *D. fortii* concentrations are as low as 200 cells·L^{-1} humans will become intoxicated (Yasumoto et al. 1980). *Dinophysis fortii* was never very abundant in the NSG during the 1986 sampling times, and only reached 200 cells·L^{-1} on one occasion in mid-Strait.

There is evidence to suggest that DSP can be caused by other species of *Dinophysis* such as *D. acuta* and *D. norvegica* (Dahl and Yndestad 1985; Freudenthal and Jijina 1985; Krogh et al. 1985; Underdal et al. 1985; Cembella 1989). In the NSG *D. norvegica* remained below 200 cells·L^{-1} during most of the summer, but did reach 500 cells·L^{-1} in surface waters of September, a possible intrusion from Sutil Channel. The smaller *D. ovum* was most abundant in June. This species has not been linked to DSP yet, although it is closely related to some that have been.

Assuming that the above four species can produce okadaic acid, it would make sense to monitor their collective distribution. These organisms posed no threat to the NSG in March and April. June and August saw concentrations exceed 200 cells·L^{-1}, especially on the west side. By September critical concentrations only occurred in the northwest, perhaps due to introduction from the northern passageways.

The silicoflagellate, *Dictyocha speculum*, has been implicated in fish kills in Danish coastal waters by Thomsen and Moestrup (1985) where a bloom (25×10^6 cells·L^{-1}) of morphologically abnormal *D. speculum* (without siliceous skeletons) occurred in May, 1983. Since then, this organism has bloomed every year in late spring immediately following the diatom bloom (Jochem and Babenerd 1989). Due to eutrophication in the Western Baltic nitrogen and phosporus are not exhausted and the phytoplankton populations become silicate-limited, allowing non-siliceous flagellates to bloom. The authors have observed blooms of *D. speculum* in various British Columbia inlets but no harmful events have been reported. In the NSG this silicoflagellate became more abundant during the summer months. In June most of the population preferred the upper 10 m, whereas in August maxima occurred between 5 and 15 m. The western side did not encourage the growth of *D. speculum* at these times.

The non-photosynthetic dinoflagellate, *Noctiluca scintillans*, grazes on whatever is present in the plankton. In the NSG it was concentrated on the western side, usually in the upper 5–10 m. In the region of the northwest tidal jet this dinoflagellate was mixed down to at least 20 m. *Noctiluca scintillans* often forms dense red water in British Columbia, with no deleterious effects.

A common theme exhibited by the potentially harmful flagellated species was that of elevated concentrations in the north and along the western side of the NSG. This distribution could have been due to: (1) stimulation by increased tidal turbulence and entrainment of nutrients, and/or (2) injection of populations from the northern passageways. The latter makes sense because there are numerous sheltered bays which could act as seed beds for cyst-forming species such as *Heterosigma* and *Protogonyaulax*.

Two flood tides enter the NSG per day, one from the north and one from the south, which meet somewhere near Cape Lazo. The flood from the north flows strongly southward through Discovery Passage along the western side until it meets the flood from the south, and the two are deflected eastwards then flow north, out through Sutil Channel and Desolation Sound (Canadian Hydrographic Service 1983). Flow is basically reversed during the ebb tide. Tully and Dodimead (1957) found that homogeneous water from the northern passages intruded the Strait during the flood tide, and stratified waters from the Strait intruded the passages during the ebb. From April to September the prevailing winds in the NSG are northwesterly (Waldichuk 1957), producing wind-driven currents which would enhance water flow from the north. Stratification patterns presented in Haigh and Taylor (1991) show clearly the

extent of the Discovery Passage tidal jet's intrusion into the NSG.

Conclusion

It appears that there are two sets of circumstances in the NSG which should be of concern to aquaculturists. The first occurs in spring, and possibly earlier, on the eastern side of the NSG where populations of *Chaetoceros convolutum* and/or *C. concavicorne* may reach harmful levels in a slightly stratified water column. Generally, conditions are not conducive to stratification at this time of year over most of the NSG, probably due to wind exposure; however, the Fraser River plume influences the eastern side year round so that surface waters are less saline.

The second critical period occurs during the summer months when thermal stratification has been established and nutrients in surface waters are low. At this time of year the western side of the NSG becomes a good location for the potentially harmful flagellated species. It seems that tidal turbulence in the north, including the Discovery Passage tidal jet, either exerts a selective force through entrainment of nutrients or injects the species in question from the northern passageways. The flagellates discussed usually preferred the upper 10 m during the day. In June *Heterosigma* dominated the NSG microplankton and possibly inhibited diatom growth (Haigh and Taylor 1991). High concentrations also occurred in the adjacent inlets to the east.

In summer, *C. convolutum* thrived between 10 and 15 m where light and nutrients were favourable to its growth. Maximum concentrations of this diatom were located in mid-Strait, possibly at the edge of the tidal jet's influence where upward turbulence would be enough to suspend it without bringing it to the surface.

There are two strategies employed by photosynthetic microplankton to ensure their continuing survival. The first is year-round persistence in the water column, exhibited by *C. convolutum* which has the low-light adaptation of chloroplasts in its hollow setae. The second strategy involves the formation of over-wintering benthic cysts (e.g. *Protogonyaulax*: Dale 1983; Anderson et al. 1984; and *Heterosigma*: Tomas 1978). The basin of the NSG is deep which is not conducive to the survival and resuspension of cysts. Perhaps areas along the margins or around islands would accommodate successful seed beds but it seems more likely that populations of cyst-forming species are allochthonous. Ideal seed bed areas appear to exist in the passageways and bays to the north.

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Measurement of Phosphorus Uptake Length in Streams: Comparison of Radiotracer and Stable PO_4 Releases[1]

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Mulholland, P. J., A. D. Steinman, and J. W. Elwood. 1990. Measurement of phosphorus uptake length in streams: comparison of radiotracer and stable PO_4 releases. Can. J. Fish. Aquat. Sci. 47: 2351–2357.

Experimental releases of radiotracer and stable PO_4 were conducted on the same or consecutive days in a temperate first-order woodland stream to determine if both techniques resulted in similar measures of PO_4 uptake length (S_w). For each set of experiments, S_w measured by radiotracer releases was substantially shorter than S_w measured by stable PO_4 releases. For stable PO_4 releases, S_w was shorter for releases resulting in small increases in PO_4 concentration in water (7–12 μgP/L) than for releases resulting in larger increases in PO_4 concentration (84–163 μgP/L). Although the uptake rate of PO_4 increased with increasing [PO_4] the increase in uptake rate was not sufficient to offset increases in downstream flux of PO_4 with stable PO_4 additions, thus resulting in longer S_w. A plot of PO_4 uptake rate against [PO_4] suggested two uptake mechanisms: biologically controlled uptake at low [PO_4] that appeared to become saturated at concentrations above approximately 5 μgP/L, and physical/chemical adsorption at high [PO_4] that continued to increase with increasing concentration. Results indicate that stable PO_4 releases will overestimate ambient S_w unless PO_4 increases are sufficiently small that they do not approach saturation of the biological community.

Du PO_4 radioactif et du PO_4 stable ont été libérés le même jour ou à un jour d'intervalle dans un cours d'eau tempéré de premier ordre traversant un boisé pour établir si ces deux produits permettent d'obtenir des mesures similaires du temps d'absorption du PO_4 (S_w). Pour chacun des essais, le S_w obtenu au moyen du radiotraceur était nettement plus court que celui obtenu avec le PO_4 stable. Dans le cas du PO_4 stable, le S_w était plus court si l'augmentation de la concentration de PO_4 dans l'eau était faible (7–12 μgP/L); avec une augmentation plus forte (84–163 μgP/L), le S_w était plus long. Bien que l'augmentation de la concentration de PO_4 s'accompagnait d'une augmentation de son taux d'absorption, ce dernier n'était pas assez élevé pour compenser les augmentations de flux de PO_4 vers l'aval dues aux additions de PO_4 stable, d'où un S_w plus élevé. Le graphique du taux d'absorption du PO_4 en fonction de la concentration de PO_4 nous permet de dégager deux mécanismes possibles de capture du PO_4 : absorption essentiellement biologique à faible concentration de PO_4, soit jusqu'à une concentration d'environ 5 μgP/L correspondant au point de saturation, et adsorption physico-chimique s'accroissant continuellement avec l'augmentation de la concentration de PO_4. Ces résultats montrent que l'utilisation de PO_4 stable donne une valeur du S_w du milieu trop élevée, à moins que l'augmentation de sa concentration n'excède pas le point de saturation de la communauté biologique.

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The concept of nutrient spiralling has been developed to describe the simultaneous processes of nutrient cycling and downstream transport in lotic ecosystems (Webster and Patten 1979; Elwood et al. 1983; Newbold et al. 1983a). A mathematical framework for quantifying nutrient spiralling has been presented (Newbold et al. 1981, 1982). Central to this framework is an index [uptake length (S_w)] that quantifies the mean distance that a nutrient atom travels from the point it enters stream water in dissolved, biologically available form (via external inputs or release from particulate forms) to the point it is taken up by particulate matter. S_w is a measure of the rate of removal of a nutrient from water relative to its downstream flux in water. Thus, S_w can be viewed as a measure of the efficiency with which stream ecosystems utilize available supplies of nutrients in water and as such is a useful comparative tool (Newbold et al. 1981, 1982). An in-situ radiotracer injection method has been developed (Newbold et al. 1981) and used to quantify differences in phosphorus spiralling between laboratory streams with different biological communities (Mulholland et al. 1983) and between seasons in a natural stream (Mulholland et al. 1985). However, the inability to perform unconfined radiotracer releases in the field due to regulatory constraints has prevented greater use of the spiralling concept as a quantitative tool in investigations of lotic ecosystems.

Alternative methods for measuring S_w for phosphorus in streams include: (1) radiotracer studies of phosphorus uptake by streambed substrates performed in recirculating chambers, combined with measurement of the downstream flux of phosphorus in stream water; and (2) whole-stream additions of stable PO_4 and a conservative (hydrologic) tracer. The chamber method permits the use of radiotracers (e.g. ^{32}P and ^{33}P) for quantifying PO_4 uptake but is limited by the ability to transfer all of the components of stream ecosystems important in phosphorus uptake into a chamber. This method is also limited by

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the accumulation of toxic material or the depletion of nutrients in the chamber water during incubation. Whole-stream additions of stable PO_4, performed over periods sufficiently short to minimize changes in ecosystem biomass or taxonomic structure, offer an attractive alternative to whole-stream radiotracer injections. However, results of experimental PO_4 additions can be used to quantify S_w only if the uptake rate of PO_4 from water increases in proportion to the increase in PO_4 concentration in water, and if the release rate of PO_4 back to water during the experimental addition is the same as the release rate prior to the addition. These considerations have been addressed in detail in a recent report (Stream Solute Workshop 1990).

The objectives of this study were: (1) to compare measures of S_w from stable and radiotracer PO_4 releases; and (2) to determine the effect of the magnitude of PO_4 enrichment by adding stable PO_4 at two concentrations. The radiotracer and stable PO_4 experiments were conducted on two occasions to determine seasonal differences: in autumn after peak leaf input when most of the uptake of PO_4 is by microbes colonizing leaf surfaces, and in summer when most of the uptake is by organisms associated with fine sediments and the epilithon (Mulholland et al. 1985). During the autumn experiment, a simultaneous addition of both PO_4 and NH_4 was also conducted to determine the effect of N:P ratios in water on short-term PO_4 uptake under enriched conditions.

Theoretical Considerations

S_w is related to the rate of uptake of nutrient from water (U, units of mass per stream bottom area per time) and the downstream flux of nutrient in water (F_w, units of mass per time):

(1) $S_w = F_w/(U \cdot w)$

where w is the stream width (Newbold et al. 1981). S_w is also related to the first-order uptake rate coefficient or turnover rate of nutrient in water (k_t, units of time^{-1}):

(2) $S_w = v/k_t$

where v is the average stream water velocity (Newbold et al. 1983a). For radiotracer P releases in which the concentration of PO_4 in water is not measurably increased, the steady-state concentration of the radiotracer at any site a distance x from the injection is given by

(3) $C(x) = C_o e^{-k_t(x/v)}$

where C_o is the radiotracer concentration at the site of the injection. Therefore, measurements of $C(x)$ at several sites along the stream, after correcting for dilution using the conservative tracer, can be plotted against distance (x) and fitted as a straight line on a semi-logarithmic scale. The slope of this line, k_t/v, is the first-order uptake rate coefficient per unit distance (k_w, units of distance^{-1}) and is the inverse of S_w (Newbold et al. 1981).

Additions of stable PO_4 can be used to estimate S_w if k_w remains constant so that the proportion of PO_4 removed per unit distance is independent of the nutrient concentration in stream water. In other words, U must increase by the same proportion as the increase in F_w (Eq. 1). As long as the volume of solution added is small relative to streamflow, then F_w is directly proportional to the nutrient concentration in stream water. Most studies of nutrient uptake by algae and bacteria indicate a Michaelis–Menten-type relationship between uptake rate (U) and nutrient concentration (C):

(4) $U = U_{max} \cdot C/K_t + C$

where U_{max} is the maximum uptake rate and K_t is the nutrient concentration at which the uptake rate is one-half U_{max}. Therefore, in order to measure S_w using PO_4 additions, concentration increases should remain within that portion of the uptake curve where U is a nearly linear function of C ($C < K_t$). Although K_t values for PO_4-limited uptake and growth determined for natural assemblages of planktonic algae or periphyton suspended in water are often <5 μg/L (Nalewajko and Lean 1980; Bothwell 1985), periphyton biomass in streams has been shown to increase with increasing PO_4 concentrations up to 28 μgP/L (Bothwell 1989), indicating that PO_4 uptake rates for intact stream periphyton communities may become saturated only at relatively high PO_4 concentrations.

In order to apply first-order tracer kinetics to calculate S_w from results of stable PO_4 and Cl additions, it is necessary to: (1) distinguish the PO_4 and Cl additions (tracers) from ambient PO_4 and Cl concentrations; and (2) conduct studies over a sufficiently short period to preclude any effect of the addition on rates of regeneration of PO_4 back to water (for additional discussion see Stream Solute Workshop 1990). The first condition can be satisfied by subtracting PO_4 and Cl concentrations measured just prior to the tracer addition from the concentrations measured during passage of the tracer pulse at each site. The second condition (no effect on regeneration ratio) can usually be met by conducting studies for periods of no more than several hours because rates of PO_4 regeneration are a function of concentration of P in organic matter rather than concentrations in water. Consequently, rates of PO_4 regeneration should not change with changes in water column PO_4 concentrations over short periods. Previous studies involving radiotracer PO_4 releases have shown that only a very small fraction of the radiotracer taken up during the release was returned to the water in the first several hours after the release (Newbold et al. 1983a; Mulholland et al. 1985).

Study Site

The study was conducted in the West Fork of Walker Branch, a first-order woodland stream located on the United States Department of Energy Oak Ridge National Environmental Research Park in eastern Tennessee. The West Fork of Walker Branch has been previously used in several studies of phosphorus spiralling involving experimental radiotracer releases (Newbold et al. 1983a; Mulholland et al. 1985). Baseflow in the stream is generated by springs and seeps that arise in dolomitic limestone. Therefore, baseflow alkalinity (2–3 meq/L) and stream water pH (7.4–8.2) are moderately high. Streamflow in the study reach is continuous, with low summer baseflows of 3–5 L/s and occasional winter and spring storm flows exceeding 50 L/s. During baseflow the stream averages about 5–10 cm in depth and about 3 m in width.

Average concentrations of soluble reactive phosphorus (SRP) and soluble inorganic nitrogen at baseflow were 3 and 27 μg/L, respectively, during 1988 and 1989 (P. J. Mulholland, unpubl. data). Past studies involving long-term phosphorus and nitrogen additions have demonstrated phosphorus limitation of periphyton growth and leaf decomposition in Walker Branch (Elwood et al. 1981b; Newbold et al. 1982). Radiotracer releases of PO_4 have indicated considerable seasonality in phosphorus cycling with the shortest phosphorus uptake lengths and hence the tightest phosphorus cycling occurring in the late autumn and early winter following leaf fall (Mulholland et al. 1985). Phosphorus uptake lengths are longest during the summer.

TABLE 1. Ambient physical and chemical conditions prior to each of the experimental releases. With the exception of water velocity, all parameters are values at the most upstream sampling station (11 m downstream from the release point).

Parameter	19 December	20 December	29 June
Streamflow (L/s)	2.7	2.7	9.4
Water velocity (cm/s)[a]	2.7	2.7	6.8
Water temperature (°C)	11.0	12.0	15.5
Specific conductance (μS/cm)	264	264	200
Chloride (mg/L)	0.7	0.7	0.7
Soluble reactive phosphorus (μg/L)	2.9	3.3	2.8
NH_4 (μg N/L)	<2.0	<2.0	8.0
$NO_2 + NO_3$ (μg N/L)	10.3	10.9	32.8

[a]Average water velocity over the 81-m reach immediately downstream from the release point. Values were determined by estimating the time for the conservative tracer to reach one-half the plateau value at station 81 m. There were insufficient measurements made during the rising limb of the injection pulse on 20 December to calculate average water velocity. Because streamflow was the same as that on 19 December, water velocity was also assumed to be the same.

Methods

Radiotracer and stable phosphorus releases were conducted on 19–20 December 1988 and 29 June 1989. In December the radiotracer and stable P releases were conducted on consecutive days. In June the radiotracer and stable P releases were conducted consecutively on the same day.

The radiotracer releases were conducted using generally similar procedures to past releases, except that $^{33}PO_4$ rather than $^{32}PO_4$ was used (Mulholland et al. 1985). A solution containing approximately 55 MBq of ^{33}P as carrier-free $H_3{}^{33}PO_4$ and 185 MBq of ^3H as tritiated water was released to the stream over a 90-min (19 December) or a 60-min (29 June) period. Tritium was added as a hydrologic tracer to correct for dilution due to groundwater inputs in the experimental stream reach. Water samples were collected at 5- or 10-min intervals at stations 11, 22, 45, and 81 m downstream from the release point. During the June releases, water samples were not collected at the 22-m station but were collected at a station 125-m downstream from the release point. Samples were collected in polyethylene syringes and filtered immediately (Whatman GF/C glass fiber filters). Samples of filtrate (5 mL) were assayed for ^{33}P and ^3H by liquid scintillation using multiple channels to separate isotopes and external standardization to correct for counting efficiency. All samples were counted within 6 h of collection to minimize differences due to decay (half-life of ^{33}P is 25.4 d). Samples were also collected at each stream station just prior to the radiotracer release for SRP and inorganic N analysis. Concentrations of SRP were measured by the molybdenum blue method (Murphy and Riley 1962). Concentrations of NH_4 were measured by automated phenate colorimetry and $NO_2 + NO_3$ by Cd-Cu reduction followed by automated colorimetry (U.S. EPA 1983).

Stable P releases consisted of two consecutive releases of a K_2HPO_4 solution: the first resulting in a low P enrichment (7.4 and 12 μg/L on 20 December and 29 June, respectively), and the second resulting in a higher P enrichment (84 and 163 μg/L on 20 December and 29 June, respectively) at the most upstream station. On 20 December a third release was conducted involving simultaneous enrichment of PO_4 (81 μgP/L) and NH_4 (370 μgN/L). For all stable P releases, NaCl was added to the release solution as a conservative (hydrologic) tracer, which resulted in increases of Cl concentration of 11–13 mg/L at the most upstream station. The low and high P releases were each for a period of 90 min on 20 December and 60 min on 29 June with a 1-h interval between them. Water samples were collected at 5- to 10-min intervals during the releases at the same stations used for the radiotracer releases. Water was filtered immediately (Gelman Type A/E glass fiber filters), placed on ice, and analyzed for SRP and Cl within 1 d of collection. Concentrations of Cl were measured by the automated ferricyanide method (U.S. EPA 1983).

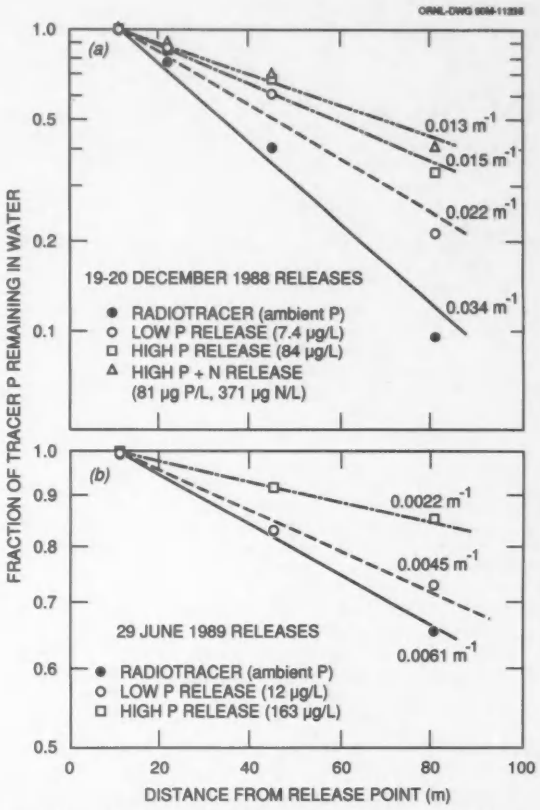

FIG. 1. Semilogarithmic plot of tracer P remaining in water with distance for the (a) 19–20 December 1988 and (b) 29 June 1989 releases. The tracer P data are presented as the fraction of the tracer remaining in water in order to allow use of one vertical scale for all releases. Fraction of tracer P remaining in water was calculated by dividing the P tracer:conservative tracer ratio ($^{33}P/^3H$ or SRP/Cl) at each station by the ratio at the most upstream site (11 m). Each ratio was calculated as the average of 8–10 values during passage of the tracer pulse at that station (CV<10% for most ratios). The slope of each line is the first-order uptake rate coefficient of PO_4 per unit distance (k_w), and its inverse is S_w.

Results

Streamflow was about 3.5-fold greater and average water velocity was about 2.5-fold greater in the June experiments than in those conducted in December (Table 1). The higher June flows resulted in a dilution of the total ionic strength of stream

TABLE 2. Uptake lengths (S_w) of PO_4 for an 81-m reach of Walker Branch measured during December 1988 and June 1989.

Release type	Uptake length of PO_4 (m)	
	19–20 December	29 June
Radiotracer	29	164
Low PO_4 enrichment[a]	45	222
High PO_4 enrichment[b]	67	455
High PO_4 and NH_4 enrichment[c]	77	—

[a]Enrichment levels: December = 7.4 μg P/L; June = 12 μg P/L.
[b]Enrichment levels: December = 84 μg P/L; June = 163 μg P/L.
[c]Enrichment levels (December only): 81 μg P/L, 371 μg N/L.

TABLE 3. Average uptake rate of PO_4 per unit stream length (U_p) under ambient conditions (radiotracer release) and with short-term PO_4 enrichments. See text for explanation of U_p calculation.

P conditions	U_p (μg P·m^{-1}·s^{-1})	
	19–20 December	29 June
Ambient	0.19	0.18
Low PO_4 enrichment	0.43	0.48
High PO_4 enrichment	2.34	2.99

water, as indicated by lower specific conductance in June than in December. Water temperatures were also higher in June than in December. In contrast to these differences in physical and bulk chemistry properties, concentrations of SRP were similar on both dates. Concentrations of inorganic nitrogen were about threefold to fourfold higher in June than in December.

Average values of k_w (the first-order uptake rate coefficient for PO_4 per unit stream length) for the upper 81 m of the study reach were higher for the radiotracer releases than for the stable P releases during the December and June experiments (Fig. 1). Moreover, k_w was higher for the low P releases than for the high P releases on each date. Simultaneous enrichment of PO_4 and NH_4 resulted in slightly lower k_w for PO_4 than did enrichment of PO_4 alone during December. A test for parallelism was performed on the regressions in Fig. 1 using the SAS GLM procedure (SAS Institute, Inc. 1985), and parallelism was rejected at the $P<0.01$ level. Statistical comparisons (Duncan's Multiple Range Test, Duncan 1970) among the slopes of the individual regressions (k_w) indicated that k_w's for the radiotracer, low P, and high P releases were statistically different ($P<0.05$) from each other in December, and k_w's for the radiotracer and low P releases were statistically different in June ($P<0.05$). Differences between k_w's for the high P and high P + N releases in December and between the low P and high P releases in June were not significant.

Concentrations of NH_4 during the high P + N release in December also declined relative to the conservative tracer over the experimental stream reach. The k_w calculated for NH_4 was 0.015 m^{-1}, slightly larger than the k_w for PO_4 (0.013 m^{-1}) for this release.

Uptake lengths of PO_4 (S_w) were shortest for the radiotracer releases and longest for the stable P releases (Table 2). The much longer S_w's measured in June compared with those measured in December are consistent with past results that indicate that S_w is longest in summer months when the abundance of leaf detritus is lowest and shortest in late autumn when leaf detritus is abundant (Mulholland et al. 1985). The seasonal pattern in S_w in Walker Branch is controlled by uptake of PO_4 from the water column by microbes involved in leaf decomposition.

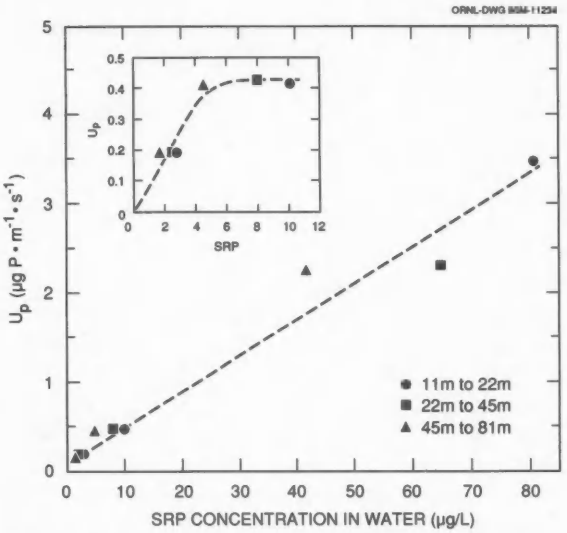

FIG. 2. Uptake rate of PO_4 (U_p) plotted as a function of SRP concentration in water measured for short stream reaches during the 19–20 December releases. The lower left corner of the plot, which consists of data from the radiotracer and low PO_4 releases, is expanded in the inset.

The average rate of PO_4 uptake per unit stream length (U_p) was calculated according to Eq. 1 as follows:

(5) $U_p = U \cdot w = F_w/S_w = F_w \cdot k_w$

with F_w estimated as the average of the measured fluxes of PO_4 at the most upstream and downstream stations in the 81-m study reach. For the stable PO_4 releases, U_p was calculated as the sum of $(U_p)_{ambient}$, determined from the radiotracer release, and $(U_p)_{enrichment}$, determined from the flux $(F_w)_{enrichment}$ and uptake coefficient $(k_w)_{enrichment}$ of the release pulse. U_p increased with PO_4 enrichment, being greatest at the highest level of enrichment for each date (Table 3). However, the increase in U_p with PO_4 enrichment was not enough to compensate for the increase in F_w and consequently the fraction of PO_4 taken up decreased (and S_w increased).

Because there was a gradient in PO_4 concentrations (and F_w) along the study reach, particularly during the stable PO_4 releases, we could determine the relationship between U_p and PO_4 concentration by calculating U_p separately for each of the shorter reaches delineated by our sampling stations. For the December releases, a plot of U_p vs. PO_4 concentration indicates that there may be two components to the relationship (Fig. 2). Results from the radiotracer and low PO_4 releases only (Fig. 2, inset) indicate that U_p may become temporarily saturated at SRP concentrations above approximately 4–5 μg/L. However, over the entire range of SRP concentrations, U_p increases with SRP, although at a slightly slower rate than at low SRP concentrations.

Discussion

S_w of PO_4 measured by performing additions of stable PO_4 should be quantitatively similar to S_w measured by radiotracer injections only if the rate of uptake of PO_4 (U_p) increases in proportion to the increase in PO_4 concentration and consequently flux (F_w). We found that U_p did not increase propor-

tionally with PO_4 concentration, even for small additions of PO_4 (7.4 and 12 μgP/L). Consequently, S_w measured for the stable PO_4 additions was substantially longer than that measured for the radiotracer injections, and this difference was greater for larger PO_4 additions (Table 2).

Although k_w calculated from the radiotracer results was substantially lower in June than it was in December (Fig. 1), U_p was only slightly lower in June than December (Table 3). Greater streamflow during the June release resulted in greater water velocity and F_w at this time, and hence a lower k_w and longer S_w. These results emphasize that k_w and S_w are indices that describe the uptake of a nutrient in relation to its downstream flux rather than the uptake per se. The similar U_p in early winter and summer also suggests a stimulation of biomass-specific PO_4 uptake by stream water velocity. In a previous study of the seasonal variation in S_w, Mulholland et al. (1985) reported that, at similar streamflows, U_p was substantially higher in late autumn than in summer because of much greater PO_4 uptake by microbes associated with decomposing leaves after autumn leaf fall. The lack of such seasonal differences in U_p observed in the present study may be attributed to higher water velocity in June than in December. Water velocity stimulation of phosphorus uptake by periphyton has been reported by others as well (Schumacher and Whitford 1965; Horner and Welch 1981) and has been attributed to reduction of boundary layer thickness. Therefore, increases in S_w with increasing streamflow may not be as large as increases in F_w because of velocity stimulation of U_p.

There is some evidence that the longer S_w's measured with stable PO_4 additions were the result of saturation of biological PO_4 uptake capacity. The relationship between U_p and SRP concentration for the radiotracer and low PO_4 releases in December may indicate saturation of U_p at SRP concentrations above approximately 4–5 μg/L (Fig. 2, inset). Bothwell (1989) reported that periphyton biomass accumulation in experimental troughs receiving water from the South Thompson River, British Columbia, increased with increasing PO_4 concentrations up to approximately 28 μgP/L. Similarly, Horner et al. (1983) found that periphyton accumulation in laboratory streams continued to increase with increasing PO_4 concentrations even at concentrations >50 μgP/L. However, these studies involved long-term growth responses of periphyton communities to added PO_4, rather than a short-term response of PO_4 uptake as measured in our study. Although periphyton communities may have the potential for large increases in PO_4 uptake with PO_4 addition as biomass accumulates and nutrient demands increase over periods of days to weeks, the short-term response to PO_4 addition is limited by the uptake kinetics of the original community and should become saturated at lower PO_4 concentrations.

The increased U_p at higher PO_4 concentrations (Fig. 2, Table 3) may have been the result of increased adsorption as more exchange sites became available as PO_4 concentration increased. Sharp increases in the stream water P:Cl ratio in June on the declining limb of the high PO_4 pulse at a station 125 m downstream from the release point indicate rapid desorption of at least some of the PO_4 taken up during passage of the pulse (Fig. 3).

Our results suggest that at low PO_4 concentrations (<5 μgP/L) biological processes with relatively high PO_4 affinity regulate PO_4 uptake but that, at higher PO_4 concentrations, biological uptake becomes P-saturated and somewhat less efficient abiotic sorption dominates. This model of biotic/abiotic control of phosphorus in streams is consistent with other observations of biologically dominated PO_4 uptake at low PO_4 concentrations (Gregory 1978; Elwood et al. 1981a) and physical/chemical control of PO_4 concentration in streams and rivers with higher PO_4 concentrations (Mayer and Gloss 1980; Hill 1982). However, in some cases, PO_4 dynamics in streams with relatively low PO_4 concentrations also appear to be controlled by physical/chemical processes (Meyer 1979; Klotz 1985).

Recent studies have demonstrated the potential importance of the hyporheic zone for nutrient uptake and retention in streams (Stanford and Ward 1988; Triska et al. 1989a,1989b). However, results from our conservative tracer releases appear to indicate that the hyporheic zone in Walker Branch is probably less important in its potential to influence stream nutrient dynamics than for the above-cited streams. Unlike the results of Triska et al. (1989a) for a third-order stream in California, steady-state concentrations of the conservative tracer were achieved relatively rapidly (within approximately 1 h) in Walker Branch (Fig. 3), indicating a small transient storage volume and suggesting a hyporheic zone with relatively small volume. This result is not surprising for small streams such as Walker Branch that have generally shallow sediments overlying bedrock.

Addition of NH_4 and PO_4 did not result in shorter S_w compared to addition of PO_4 alone in December, despite the relatively low ambient N:P molar ratio (7.8) in stream water at that time. Thus, uptake of PO_4 was not limited by N availability, at least during the high PO_4 addition conducted in December. However, most of the PO_4 uptake may have been the result of physical/chemical processes at high PO_4 concentrations, and the effects of added N on biological PO_4 uptake may have been obscured. In studies involving long-term additions of N and P to streams, the growth response of periphyton communities to combined N and P addition is often greater than the response to the addition of either nutrient alone (Stockner and Shortreed 1978; Crawford 1979; Peterson et al. 1983; Triska et al. 1989a). These results suggest that additions of either N or P alone can result in a switch of the nutrient limiting growth and consequently the uptake of all other essential nutrients. However, for an experimental release of the limiting nutrient lasting only several hours, increased uptake of the limiting nutrient may not result in increased metabolic demands for other nutrients.

Our results suggest caution when using stable element additions for quantifying nutrient uptake lengths in streams. We have shown that when nutrient concentrations are increased well above background levels, the nutrient uptake lengths calculated are not representative of ambient conditions. Our results do suggest that if stable nutrient releases cause relatively small increases in concentrations, uptake lengths will be similar to ambient values. For Walker Branch, increases in PO_4 concentration would have to be limited to about 2–3 μgP/L to maintain ambient PO_4 uptake lengths. However, analytical precision of PO_4 measurement (±0.5 μgP/L) generally requires larger PO_4 enrichments. Perhaps somewhat higher PO_4 enrichments could be used in streams with higher potential for PO_4 uptake due to more developed epilithic communities or greater accumulations of detritus than in Walker Branch. Regardless, if stable element releases (enrichments) are to be used for estimation of nutrient uptake lengths, then whole-stream uptake kinetics should be determined for a range of nutrient concentrations to ensure that biological uptake is not saturated with the nutrient additions (Stream Solute Workshop 1990).

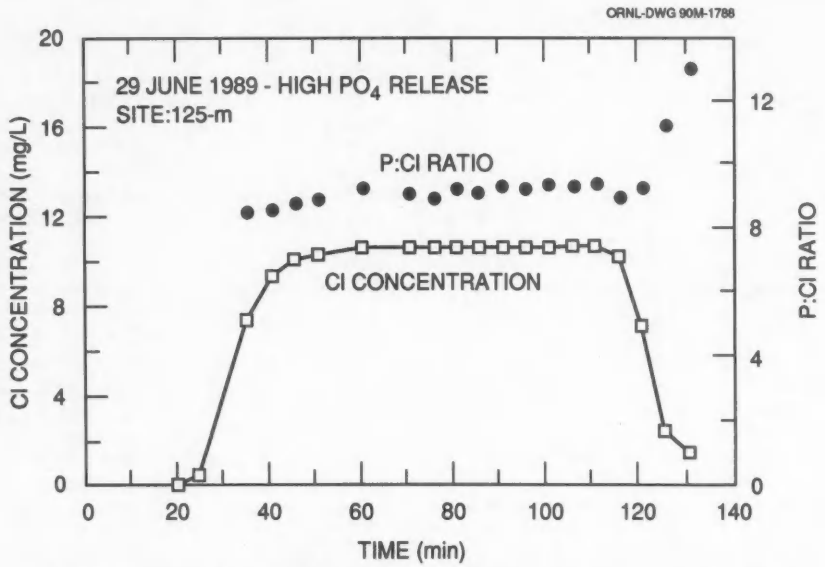

FIG. 3. Cl concentration (□) and P:Cl concentration ratio (●) plotted over time at a site 125 m downstream from the release point for the high PO_4 release on 29 June 1989.

These considerations do not preclude the use of stable element releases for estimation of nutrient uptake *potential* in streams, however. This information may be valuable for comparing stream response to disturbance or providing estimates of maximum uptake rates.

Releases of carrier-free radiotracers are the preferred method for measuring nutrient uptake lengths in streams. Although there is reluctance to use radiotracers in natural systems, regulatory barriers may not be insurmountable, particularly for using isotopes such as ^{33}P, which has a relatively short half-life (25 d) and relatively low energy (0.248 MeV). Alternatively, $^{33}PO_4$ or $^{32}PO_4$ could be used in chambers into which stream bottom communities and stream water are placed to measure PO_4 uptake rates at ambient PO_4 concentrations. S_w could then be calculated using Eq. 1 if the flux of PO_4 in stream water was also measured.

Acknowledgements

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Physiological Responses of Largemouth Bass, *Micropterus salmoides*, Exposed to Salinity[1]

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Meador, M. R., and W. E. Kelso. 1990. Physiological responses of largemouth bass, *Micropterus salmoides*, exposed to salinity. Can. J. Fish. Aquat. Sci. 47: 2358–2363.

Plasma osmotic and electrolyte concentrations as well as branchial Na^+/K^+ and Mg^{++} ATPase activities were determined in the field for largemouth bass, *Micropterus salmoides*, from a brackish marsh and freshwater lake in southcentral Louisiana. Laboratory experiments were conducted to evaluate plasma chemistry and gill ATPase activities of largemouth bass from both locations exposed to 0, 4, 8, and 12‰ salinity. No significant differences in physiological responses were detected between marsh and freshwater largemouth bass exposed to 0, 4, or 12‰. Exposure to 12‰ salinity resulted in osmotic stress in largemouth bass from both locations. At 8‰, marsh largemouth bass had significantly higher plasma solutes and lower gill ATPase activities than freshwater fish. Different physiological responses by marsh and freshwater largemouth bass during exposure to 8‰ salinity indicated that marsh largemouth bass have adapted to environments of variable salinity by reducing active ion transport and tolerating elevated plasma ion levels.

La pression osmotique et les concentrations des électrolytes dans le plasma de même que l'activité de la Na^+/K^+ ATPase et de la Mg^{++} ATPase dans les branchies ont été déterminées chez des achigans à grande bouche, *Micropterus salmoides*, provenant d'un marais d'eau saumâtre et d'un lac d'eau douce du centre-sud de la Louisiane. La chimie du plasma et l'activité des ATPases au niveau des branchies ont été étudiées chez les deux groupes d'achigans qui ont été exposés à des salinités de 0, 4, 8 et 12‰. Aucune différence significative n'a été observée dans les réponses physiologiques des achigans de marais et d'eau douce pour les salinités de 0, 4 et 12‰. À 12‰, les achigans des deux provenances ont subi un stress osmotique. À 8‰, les achigans de marais montraient des concentrations de solutés plasmatiques supérieures et des activités branchiales ATPasiques inférieures à celles des achigans d'eau douce. Les réponses physiologiques différentes observées chez ces deux groupes d'achigan lors de leur exposition à une salinité de 8‰ montrent que les achigans de marais se sont adaptés aux variations de salinité de leur milieu en réduisant le transport actif des ions et en développant une tolérance aux concentrations élevées d'ions dans leur plasma.

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Many species of freshwater fishes have been collected in low-salinity marshes along the Atlantic and Gulf coasts of the United States (Hackney and de la Cruz 1981; Rozas and Hackney 1984). Among these is the largemouth bass, *Micropterus salmoides*, a recreationally important gamefish. Although Renfro (1959) indicated that chronic exposure to salinities above 9‰ were lethal to largemouth bass, this species has been collected at salinities as high as 24‰ (Bailey et al. 1954).

Largemouth bass that inhabit brackish environments must tolerate sometimes sudden and drastic fluctuations in salinity, yet there is little information concerning the effects of salinity on largemouth bass physiology. The ability of largemouth bass to inhabit environments of variable salinity is dependent on ionoregulatory mechanisms that maintain proper osmotic balance. Gill ATPase activities, including Na^+/K^+ ATPase and Mg^{++} ATPase, have been shown to play an important role in ion regulation and saltwater adaptation of freshwater euryhaline fishes (Epstein et al. 1967; Kamiya and Utida 1969; Towle et al. 1977). Inability of freshwater fishes to adapt to increased salinity results in increased electrolyte concentrations and osmotic stress (Lutz 1972; Davis and Simco 1976; Furspan et al. 1984).

Comparative studies of largemouth bass from freshwater and low-salinity habitats indicated significant differences in morphology, growth rate, and salinity preference (Meador 1988; Meador and Kelso 1989). In the present study, we examined the variability in physiological responses to salinity between largemouth bass from the two habitats. As a measure of physiological response to differing salinities, we examined plasma chemistry and gill ATPase activities of largemouth bass from a brackish marsh and freshwater lake. Plasma osmolality and electrolyte concentrations were used as indicators of osmoregulatory dysfunction.

Methods

We collected marsh largemouth bass from canals near Cutoff, Louisiana, USA (29° 30′ N, 90° 10′ W) in October 1987. Although salinities in the canals vary seasonally and can range from 0 to 12‰ over a year (Meador and Kelso 1989), salinities

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TABLE 1. Plasma osmotic and electrolyte concentrations (mean ± SD and range) of marsh and freshwater largemouth bass in the field collected at 0‰ salinity and held at 0‰ in the laboratory. Normal ranges are ± 2 SD of mean pooled values.

		Osmolality (mOsm/L)	Na^+ (meq/L)	Cl^- (meq/L)	K^+ (meq/L)
		(N = 12 per location)			
Field	Marsh	311 ± 6.4	127.9 ± 36.4	101.2 ± 4.2	2.8 ± 0.7
		297–320	96.7–180.0	92.5–110.6	1.9–4.2
	Freshwater	306 ± 6.7	114.7 ± 23.4	99.9 ± 4.9	2.7 ± 0.5
		297–321	95.0–171.0	94.6–112.7	1.9–3.6
	Normal ranges	295–323	60–183	91–110	2–4
		(N = 4 per location)			
Laboratory	Marsh	311 ± 7.9	127.9 ± 15.3	94.0 ± 4.1	2.3 ± 0.6
		299–316	113.1–147.1	88.1–97.3	1.4–2.8
	Freshwater	310 ± 1.7	127.2 ± 11.3	96.4 ± 2.0	3.0 ± 0.4
		308–312	112.8–138.9	94.3–99.1	2.6–3.5

were 0‰ when collections were made. Freshwater largemouth bass were collected from False River (30° 40′ N, 91° 30′ W), an 860-ha oxbow lake of the Mississippi River, during the same period. Adult largemouth bass (215–361 mm total length) were sampled from both locations by electroshocking, which has been reported to have insignificant effects on blood chemistry values of largemouth bass (Burns and Lantz 1978).

Field

Twelve fish from each study location were collected and osmolality, electrolyte, and ATPase values were taken from each individual. Within minutes of collection, each fish was anesthetized with tricaine methanesulfonate. Blood was collected in lithium-heparinized Vacutainer tubes (Becton Dickinson, Vacutainer Systems) from vessels in the caudal peduncle, transferred to 1.5 mL centrifuge tubes, and centrifuged for 2 min at 13,000 *g* centrifugal force; plasma was subsequently removed with a disposable pipette. Plasma osmolality values were determined immediately after centrifugation using a 50-µL sample in a freezing-point depression osmometer (Precision Systems, Model 5004). Remaining plasma was retained for analysis of ion content, and was placed on dry ice, transported to the laboratory, and stored at −77°C in an ultracold freezer. Gill filaments were trimmed from gill arches, placed on dry ice, transported to the laboratory, and stored at −77°C for analysis of gill Na^+/K^+ ATPase and Mg^{++} ATPase activity. Sodium and potassium levels were determined simultaneously using inductively coupled plasma (gas) atomization (Applied Research Laboratories, Model 34000). Chloride concentrations were determined with a chloridometer (Haake Buchler, Model 4425), and branchial ATPase activities were determined by the method of Zaugg (1982). All fish were collected and osmolality values processed at approximately the same time of day.

Laboratory

Largemouth bass from each location were transported to the laboratory and held in aerated fiberglass tanks (about 10 fish per tank) containing 305 L of biofiltered tapwater at 0‰. Nitrite and ammonia levels monitored once a week did not exceed 0.1 mg/L. All fish were allowed 2 wk to recover from capture stress and fed live bluegill, *Lepomis macrochirus*, ad libitum daily. Tank water was maintained at a temperature of 22°C under a 14 h light : 10 h dark photoperiod. After the 2-wk holding period, four largemouth bass per location at 0‰ were removed and sampled for plasma chemistry and gill ATPase activities as previously described. Salinity was then increased 1‰ per day to 4‰ using an artificial sea salt solution (Instant Ocean), measured by refractometer or salinometer. Four fish from each location were removed and sampled for plasma chemistry and gill ATPase activities at 6, 12, 24, 72, and 120 h of exposure. After 120 h of exposure to 4 ppt salinity, salinity was increased 1‰ per day to 8‰ and sampling after the five exposure times repeated. Salinity was then increased as before to 12‰, and sampling after the five exposure times repeated. In addition, three fish from each location were sampled at 336 h of exposure to 12 ppt.

All data were analyzed using the general linear model procedure of SAS (SAS Institute Inc. 1985). ANOVA was used to evaluate differences in plasma chemistry values between locations and exposure times. Gill ATPase activities were compared across salinity treatments by a Student–Newman–Keuls multiple range test. Statistical significance was declared at the $P \leq 0.05$ level.

Results

Field Studies

No significant differences were detected in plasma chemistry values between marsh and freshwater fish (all $P > 0.05$). The mean of each plasma variable ± 2 SD has been used to define 'normal' plasma values for largemouth bass (Carmichael et al. 1984). Therefore, data from both locations were combined to establish normal plasma osmotic and electrolyte values (Table 1). Plasma chemistry values observed during laboratory trials were compared to these normal ranges. Osmotic stress was determined by values exceeding the upper limit of this normal range.

No differences in gill Na^+/K^+ ATPase ($P = 0.60$) or Mg^{++} ATPase activity ($P = 0.17$) were detected between marsh and freshwater largemouth bass. Mean gill Na^+/K^+ ATPase activities of marsh and freshwater largemouth bass were 3.5 µmolP_i·mg protein^{-1}·h^{-1} ± 1.3 SD (range 1.4–5.9) and 3.8 ± 1.8 µmolP_i·mg protein^{-1}·h^{-1} (range 0.8–7.6). Mean gill Mg^{++} ATPase activity of marsh largemouth bass was 6.9 µmolP_i·mg protein^{-1}·h^{-1} ± 2.4 SD with a range of 4.1–11.3. Values for freshwater largemouth bass ranged from

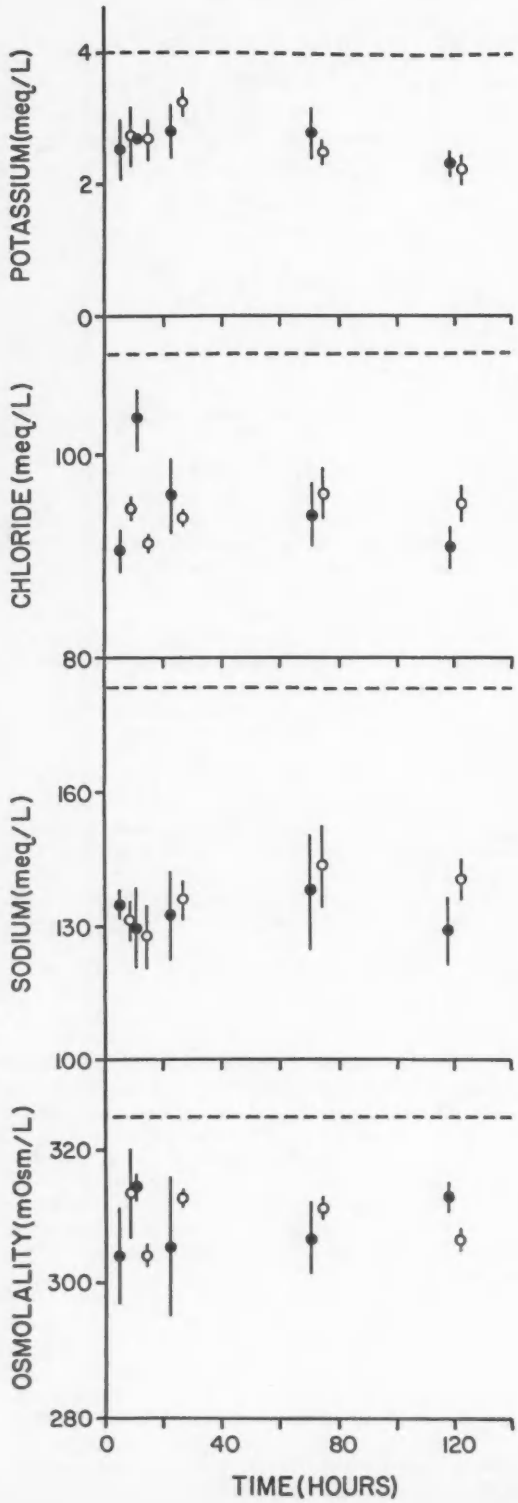

FIG. 1. Plasma osmolality and electrolyte concentrations (mean ± SD) of largemouth bass from a brackish marsh (●) and freshwater lake (○) at 6, 12, 24, 72, and 120 h exposure to 4‰ salinity. Mean values are based on four fish for all exposure levels. Dashed lines represent upper limit of normal values for each plasma concentration.

3.1–12.7 $\mu molP_i \cdot mg\ protein^{-1} \cdot h^{-1}$ with a mean of 8.2 ± 3.5 SD.

Laboratory Studies

No differences in plasma osmotic or electrolyte concentrations were detected between marsh and freshwater largemouth bass held at 0‰ (all $P > 0.05$), and mean plasma chemistry values were within field-based normal ranges (Table 1). Similarly, no differences in plasma chemistry were detected at 4‰ (all $P > 0.05$ for location and time; Fig. 1). However, at 8‰, plasma solute levels were significantly greater for marsh fish (all $P < 0.05$ between locations; Fig. 2). Variability in plasma concentrations with increasing exposure time to 8‰ was found for chloride ($P = 0.01$) and potassium ($P = 0.02$), although no clear pattern was observed. No other differences in plasma chemistry were detected with duration of exposure to 8‰ salinity ($P > 0.05$). All mean plasma chemistry values were within normal ranges during exposure to 4 and 8‰ (Fig. 1, 2).

Plasma osmotic and electrolyte concentrations increased significantly with duration of exposure to 12 ‰ ($P < 0.05$), but no differences in plasma chemistry were detected between marsh and freshwater largemouth bass at this salinity (all $P > 0.05$, Fig. 3). Fish from both locations stopped feeding within 48 h of exposure to 12‰ and mean plasma chemistry values exceeded the upper limit of the normal range during exposure to 12‰ (Fig. 3). Chloride values exceeded the upper limit of the normal range within 6 h of exposure to 12‰ whereas sodium and potassium values did not exceed this level until 24 h of exposure.

No differences in gill ATPase activities were detected with duration of exposure to a given salinity. Therefore, all data within a given salinity were combined to evaluate the influence of salinity on gill Na^+/K^+ and Mg^{++} ATPase activities of largemouth bass from each location. No differences in gill Na^+/K^+ ATPase or Mg^{++} ATPase activity were detected between marsh and freshwater largemouth bass held at 0, 4, or 12‰ ($P > 0.05$, Table 2). However, freshwater largemouth bass exhibited higher gill Na^+/K^+ ($P = 0.0002$) and Mg^{++} ATPase ($P = 0.0002$) activities at 8‰ (Table 2).

No differences in mean gill ATPase activities of marsh largemouth bass were detected among salinity levels ($P = 0.49$). Mean gill ATPase activities of freshwater largemouth bass were not different among 0, 4, and 12‰ ($P > 0.05$). However, gill Na^+/K^+ and Mg^{++} ATPase activities of freshwater fish were greater at 8‰ ($P = 0.01$; Table 2).

Discussion

Capture, handling, and anesthesia are reported to have profound effects on plasma osmotic and electrolyte levels in fishes (Eddy 1981; Robertson et al. 1987). However, normal ranges of largemouth bass plasma values established from field data in the present study were similar to normal values previously reported for largemouth bass (Carmichael et al. 1984; Canfield et al. 1985; Williamson and Carmichael 1986). Although gill

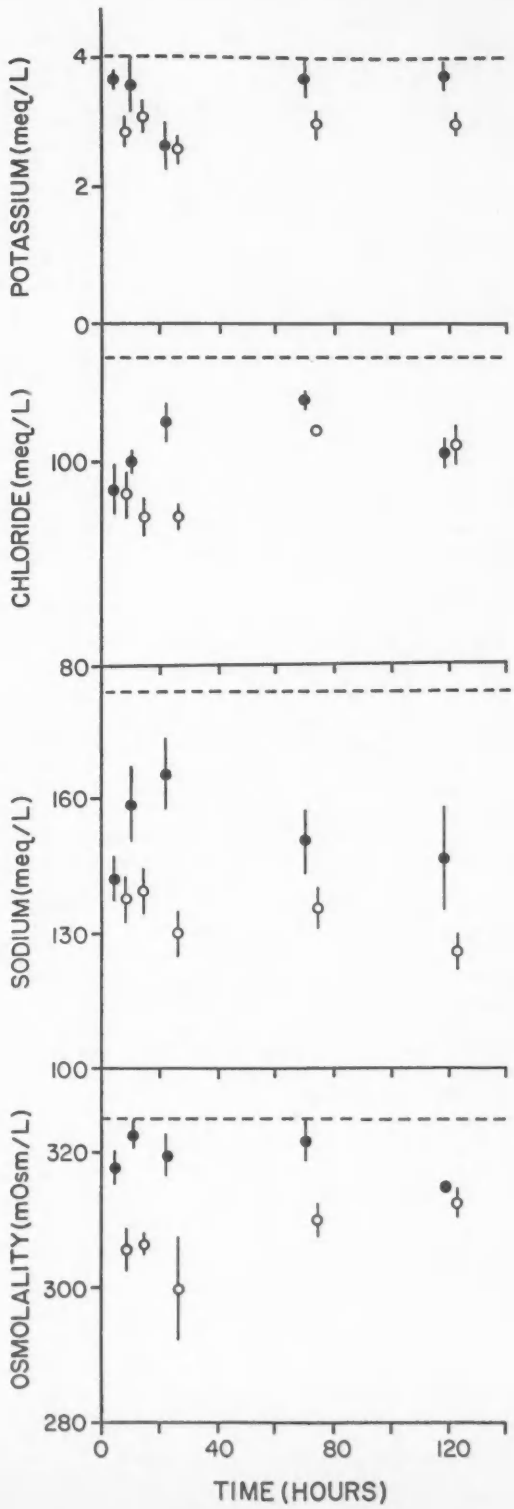

FIG. 2. Plasma osmolality and electrolyte concentrations (mean ± SD) of largemouth bass from a brackish marsh (●) and freshwater lake (○) at 6, 12, 24, 72, and 120 h exposure to 8‰ salinity. Mean values are based on four fish for all exposure levels. Dashed lines represent upper limit of normal values for each plasma concentration.

Na^+/K^+ and Mg^{++} ATPase activities have not been characterized for largemouth bass, Jampol and Epstein (1970) indicated that gill Na^+/K^+ ATPase activities of smallmouth bass *Micropterus dolomieui* ranged from 1.0 to 6.0 $\mu molP_i \cdot mg\ protein^{-1} \cdot h^{-1}$, while Mg^{++} ATPase activities ranged from 11.5 to 15.4. Whereas Mg^{++} ATPase activities in the present study were generally lower than those reported for smallmouth bass, Na^+/K^+ ATPase activities in this study were in close agreement with those noted by Jampol and Epstein (1970). Therefore, laboratory observations were assumed to reflect relative physiological responses of freshwater and marsh largemouth bass exposed to salinity.

Experiments designed to examine growth of largemouth bass from a brackish marsh and freshwater lake demonstrated that largemouth bass from both populations died within 120 d of exposure to 12‰ salinity (Meador 1988). Results of laboratory trials in the present study indicated that exposure of marsh and freshwater largemouth bass to 12‰ can initiate osmotic stress within 6 h. Most freshwater teleost fishes maintain a blood osmolality of 265–325 mOsm (Bond 1979) suggesting that they are isosmotic with 8–9 ‰ salinity. Many freshwater species, including catfishes *Ictalurus* spp. and perch *Perca fluviatilis* are reported to have difficulty osmoregulating in salinities greater than 10‰ (Lutz 1972; Furspan et al. 1984). Exposure to a hypertonic medium results in osmoregulatory failure in largemouth bass from both populations as ion concentrations, primarily Cl^-, increase.

In our study, largemouth bass responded quickly to changes in salinity. Lack of significant differences with duration of exposure to salinities of less than 12‰ suggested that largemouth bass adjusted to increasing salinity within 6 h of exposure. Peterson (1988) reported differences in salinity acclimation periods among centrarchids ranging from 1 h to greater than 12 h. However, Peterson (1988) transferred fish abruptly to varying salinities, whereas in the present study, we gradually adjusted fish to test salinities. Differences in these experimental designs and their effect on physiological responses of centrarchids are unknown.

The influence of salinities of 4‰ or less on largemouth bass plasma values was minimal. Largemouth bass have been shown to reproduce in salinities up to 4.5‰ (Tebo and McCoy 1964). Peterson et al. (1987) reported that salinities of 2–4‰ were metabolically less costly to *Lepomis* spp. than 8‰. Therefore, largemouth bass may osmoregulate efficiently at salinities less than or equal to 4‰ due to reduced energetic costs.

Differential physiological responses to 8‰ were observed between marsh and freshwater largemouth bass, with marsh fish exhibiting significantly higher plasma chemistry values and lower gill ATPase activities than freshwater fish. These results suggested that marsh largemouth bass conserved ATP and thereby reduced active ion transport at 8‰. Research has indicated that changes in gill permeability resulting in water conservation may be more important than active ion transport in fishes (Nordlie and Leffler 1975; Leray 1984). Peterson (1988) suggested that the centrarchid *Lepomis microlophus* osmoregulated consistently across salinities up to 8‰ possibly due

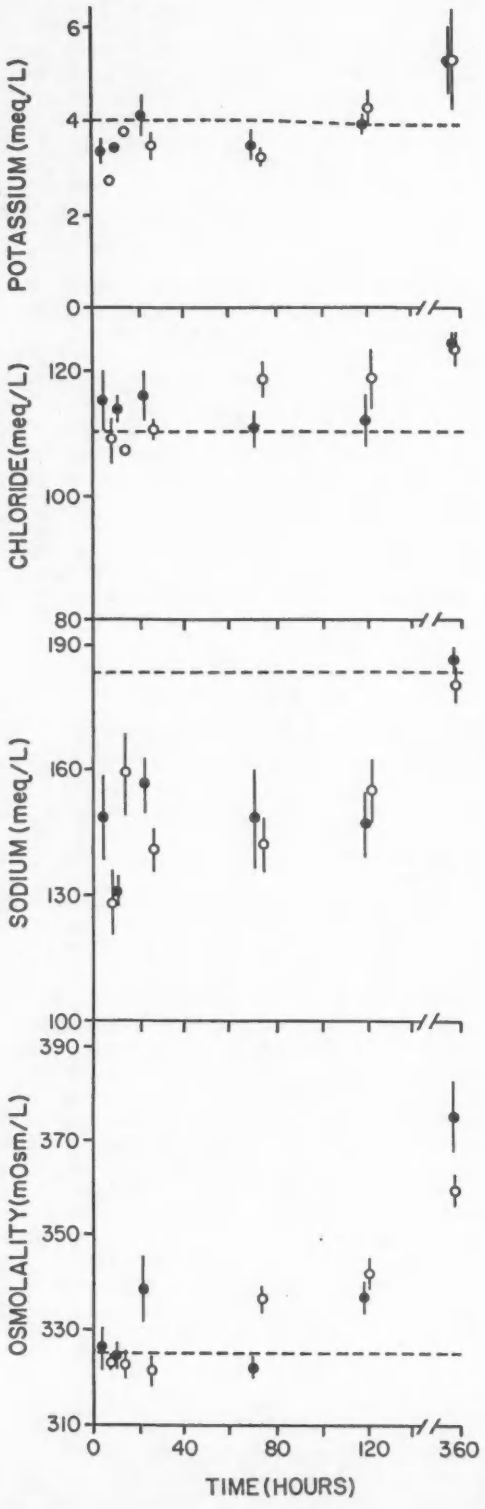

TABLE 2. Mean (± SD and range) gill Na^+/K^+ and Mg^{++} ATPase activities of largemouth bass from a brackish marsh and freshwater lake exposed to 0, 4, 8, and 12‰ salinity. Values are based on 20 individuals per location except 12‰ ($N = 23$). Data are presented as $\mu molP_i \cdot mg\ protein^{-1} \cdot h^{-1}$. Means with the same letter are not significantly different.

	Na^+/K^+ ATPase		Mg^{++} ATPase	
Salinity	Marsh	Freshwater	Marsh	Freshwater
0	3.2 ± 2.4^a	3.2 ± 1.3^a	6.9 ± 2.5^a	5.8 ± 1.6^a
	1.3–6.6	1.9–4.9	4.9–10.5	3.9–7.6
4	2.6 ± 1.3^a	3.3 ± 2.0^a	7.1 ± 2.4^a	7.3 ± 1.9^a
	1.0–5.4	0.7–8.8	4.8–11.7	4.1–11.9
8	3.5 ± 1.9^a	6.0 ± 2.7^b	7.1 ± 2.0^a	13.3 ± 5.8^b
	1.0–8.7	2.8–12.5	4.9–11.9	6.2–26.6
12	3.1 ± 1.9^a	3.9 ± 2.0^a	8.4 ± 2.6^a	9.1 ± 3.8^a
	1.06–6.2	0.9–7.6	4.1–14.7	3.5–18.8

to a reduction in the amount and location of blood flow through the gills. Such a reduction in blood flow could prevent excessive exposure of blood to sites of ion and water exchange. Therefore, differences in plasma concentrations between marsh and freshwater largemouth bass may be due to factors related to water conservation such as changes in gill surface area or permeability.

Differences in gill ATPase activities between marsh and freshwater largemouth bass were observed across salinity treatments. Whereas no differences in gill ATPase activities of marsh fish were detected among salinity levels, gill ATPase activities of freshwater largemouth bass were greatest at 8‰. Stagg and Shuttleworth (1982) indicated that the lowest activity of Na^+/K^+ ATPase occurs where ion transport is lowest. In theory, this should be at the isosmotic point, about 9‰ for largemouth bass. However, Peterson et al. (1987) and Peterson (1988) reported substantial osmoregulatory energy expenditures in centrarchids at 8‰. Towle (1984) reported that Na^+/K^+ ATPase activities in some teleost fishes may not demonstrate easily-interpretable responses to salinity change, and this appears to be true for largemouth bass.

The distinct pattern of physiological responses at 8‰ salinity suggested that marsh largemouth bass have developed osmoregulatory mechanisms in response to the brackish coastal environment in Louisiana. These largemouth bass appear to have adapted to low-salinity environments by reducing active ion transport and tolerating higher plasma levels. Although this study was designed to evaluate physiological responses to static salinity concentrations, coastal marshes are dynamic and exhibit substantial fluctuations in salinity levels over short periods. Largemouth bass in coastal marshes may conserve energy and water loss while tolerating short-term elevated plasma levels as salinities fluctuate. However, an understanding of the significance of such physiological variation will require identification of branchial and renal osmoregulatory mechanisms related to water conservation under fluctuating salinity conditions, as well as the impact of elevated plasma concentrations on growth and reproduction of largemouth bass.

FIG. 3. Plasma osmolality and electrolyte concentrations (mean ± SD) of largemouth bass from a brackish marsh (●) and freshwater lake (○) at 6, 12, 24, 72, 120, and 336 h exposure to 12‰ salinity. Mean values are based on four fish for all exposure levels except values at 336 h which were based on three fish. Dashed lines represent upper limit of normal values for each plasma concentration.

Acknowledgments

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Approaches to Age-Structured Separable Sequential Population Analysis

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Modern statistical methods are being used more often to perform age-structured separable sequential population analysis (SSPA). This paper describes how some of these methods can be easily understood from a unified point of view. The approach is to begin with the now standard separable age-structured model, and modify some of the basic assumptions. The resulting models are examined using Monte Carlo simulation, with the mean square error of modeled biomass estimates used as the evaluation criterion. Simulation results indicate that nonlinear least squares and multinomial maximum likelihood are both capable of fitting lognormally and multinomially distributed catch-at-age data. It also appears that errors in modeling results introduced by ageing error may be minor, provided ageing error is of modest magnitude and is normally distributed. However, use of a somewhat incorrect functional form for the selectivities can cause greatly increased error in the modeling results, indicating that caution should be exercised when modeling selectivities. Results indicate that length-based SSPA is feasible. And finally, the models are used to provide insight into the old question of "how many fish should be aged?"

Des méthodes statistiques modernes sont utilisées plus souvent pour effectuer une analyse séquentielle de population séparable selon l'âge. Le présent article décrit comment il est possible de comprendre facilement certaines de ces méthodes d'un point de vue unifié. D'abord, il s'agit de modifier certaines des hypothèses de base du modèle séparable selon l'âge maintenant normalisé. Les modèles ainsi obtenus sont étudiés à l'aide de la simulation de Monte Carlo, l'erreur quadratique moyenne des estimations modélisées de la biomasse servant de critère d'évaluation. Les résultats de la simulation montrent que des moindres carrés non linéaires et le maximum de vraisemblance multinomial sont deux méthodes pouvant ajuster les données sur les prises selon l'âge à distribution log-normale et multinomiale. Il semble aussi que des erreurs de modélisation des résultats introduites par suite d'une erreur de détermination de l'âge peuvent être de peu d'importance, pourvu que cette erreur soit modeste et à distribution normale. Toutefois, l'utilisation d'une forme fonctionnelle quelque peu incorrecte pour les sélectivités peut augmenter considérablement l'erreur des résultats de modélisation; il faut donc user de prudence lorsque l'on modélise des sélectivités. D'après les résultats, une analyse séquentielle de population séparable en fonction de la longueur peut être effectuée. Et en dernier lieu, les modèles permettent de résoudre l'éternelle question qui est de savoir chez combien de poissons il faut déterminer l'âge.

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Age-structured separable sequential population analysis (SSPA) has become an accepted method for analyzing catch-at-age data in fisheries stock assessment work. By SSPA, I am referring to the modeling of cohort abundance using the standard exponential survival and catch equations which have usually been attributed to Baranov (1918), plus the modeling of instantaneous fishing mortality using the "separability" assumption (J. G. Pope, unpubl. data). The complete SSPA model appears to have been first formulated by Doubleday (1976).

Fournier and Archibald (1982) made several original contributions which I apply in this paper. These include the use of the multinomial likelihood function rather than least squares in the estimation process, the general notion that auxiliary information can be incorporated into the likelihood function, and the use of an ageing error matrix.

Although the papers by Doubleday (1976) and Fournier and Archibald (1982) are the most fundamental, the approach used in this paper was also greatly influenced by Deriso et al. (1985) who followed Doubleday's (1976) least squares line of modeling, and Methot (1986) who followed Fournier and Archibald's (1982) multinomial likelihood line of modeling. While Doubleday (1976) and Fournier and Archibald (1982) suggested specialized algorithms to estimate parameters, Deriso et al. (1985) and Methot (1986) chose a simpler approach using general nonlinear least squares and function minimizing software, respectively. Although this difference may seem minor, the result is a theory that is far simpler and therefore more likely to be used.

In the context of SSPA, the most important technical contribution of Deriso et al. (1985, 1989) was largely to do for Doubleday's (1976) model what Fournier and Archibald (1982) did earlier for the multinomial likelihood model: provide a framework in which auxiliary information such as fishing effort and stock-recruitment relationships could be systematically used to constrain the model.

Fournier and Archibald (1982) held the view that models should be very flexible: "Finally, an important point should be emphasized which is too often overlooked in the design or application of models for analyzing fisheries data. When attempting to apply an existing model to a new set of fisheries data, one almost invariably encounters some new contingency

which calls for major or minor revisions to the model. For this reason a model should not be regarded as a final product but rather as a flexible framework which can always be modified should the situation require it." Methot (1986) and Methot and Hightower (1988), championed this view, showing how a model could be tailored to available survey age and/or length data by modeling selectivities for all of the different fishing gears.

Although the philosophy of tailoring a flexible model to all available data has some obvious advantages, it may also cause problems. The goal of the analysis should be to draw inferences from the data, not necessarily to "model" the process. When adding detail to a model, the modeler is presented with many possible modeling options that can significantly affect the outcome in ways that are difficult to foresee. Also, adding too much detail to a model may be akin to statistically overfitting the data and thus increasing variability in the inference. Since leaving out important detail in a model may introduce bias, even while it reduces variance (Kimura 1989), the question of how much detail should be included in a model is difficult to answer.

The goal of this paper is to derive and examine a class of SSPA models that incorporate the following key assumptions: ageing may provide either correct ages or ages with error, the data may consist of either observed age or length compositions, and the fitting criterion may be either nonlinear least squares or multinomial maximum likelihood. Thus, this paper deals with a class of eight possible models. Although it is hoped that we bring some new insights to these models, it should be emphasized that this paper is based on models and approaches previously derived by the cited authors.

An examination of these models using Monte Carlo simulation allows us to determine their robustness to misspecifications. For example, simulating catch-at-age data using lognormal and multinomial distributions, and then analyzing the data using nonlinear least squares and multinomial maximum likelihood estimation gives some indication as to the similarity and robustness of these models. In a similar vein, samples can be generated incorporating ageing error, and parameters can be estimated ignoring ageing error, in order to examine how ageing error might affect modeling results. Also, age selectivities can be modeled (Fournier and Archibald 1982; Deriso et al. 1985; Methot 1986) and the results examined when the functional form that is used is either correct or incorrect. Another interesting possibility is to simulate length-based age-structured SSPA. And finally, simulating multinomial age samples using different sample sizes, and then analyzing the variability in the modeling results, provides yet another way of examining the old question of "how many fish should be aged?"

Separable Sequential Population Analysis

Let $i=1,\ldots,y$ be the year index, and $j=1,\ldots,a$ be the age index for some species which has a nonzero ages of catch. Let

c_{ij} = the observed catch in numbers at age,

F_i = the instantaneous fishing mortality rate for fully available ages (i.e. ages for which $s_j = 1$),

s_j = the selectivity for age j fish such that the assumption of "separability" holds, i.e.

$F_{ij} = F_i s_j$
= the instantaneous fishing mortality rate of age j fish during year i.

Note also that N_{ij} = the total population numbers at age,

$U_{ij} = F_{ij}(1\text{-}\exp(-M-F_{ij}))/(M+F_{ij})$
= the exploitation rate on age j fish in year i, assuming an instantaneous natural mortality rate of M.

It follows that

$N_{i+1,j+1} = N_{ij}\exp(-M-F_{ij})$, and predicted catch would be $\hat{c}_{ij}=U_{ij}N_{ij}$. Assuming the annual instantaneous natural mortality rate M is known, this model contains $p = 2y+2a-1$ unknown parameters:

$$b=\{F_1,\ldots,F_y,s_1,\ldots,s_a,N_{11},\ldots,N_{1a},N_{21},\ldots,N_{y1}\}$$

with $m = ya$ observations. These quantities and equations are the core of SSPA. All of the methods discussed in this paper use this core model. Quantities estimated from this model and used in the model fitting algorithm will be denoted with "hats" (e.g. $\hat{c}_{ij}$).

When selectivities are individually estimated, I call them "Doubleday" selectivities; but as mentioned earlier the selectivities may be modeled as a function of age [i.e. $s_j=f(j)$]. A critical constraint when using Doubleday selectivities is that at least one of the selectivities should be fixed equal to one (i.e. $s_j=1$ for at least one value of j). In an earlier paper (Kimura 1989), I considered the problem of constraining, and testing constraints on selectivities for this model.

As noted earlier (Kimura 1989), for reliability in the estimation process it is almost always desirable to estimate the log-parameters rather than parameters on the original scale. This can be seen to make the parameters more similar in magnitude, and probably reduces parameter-effects nonlinearity (Ratkowsky 1983).

Parameters can be estimated by making assumptions about the distribution of catch-at-age. If we assume that catches (in numbers) at age are distributed as lognormal random deviates, then the maximum likelihood estimates are found by minimizing the residual sum of squares:

$$\text{SSC} = \Sigma_{ij}(\log(c_{ij})\text{-}\log(\hat{c}_{ij}))^2.$$

The model up to this point was described rather completely by Doubleday (1976). We shall call this the "nonlinear least squares" model (NLS). In a previous paper (Kimura 1989) which considered this model from a statistical point of view, I referred to this model as the Doubleday-Deriso model.

A major contribution for this class of model came when Fournier and Archibald (1982) published their paper on multinomial maximum likelihood estimation. Fournier and Archibald argued that catch at age data are more logically modeled with a multinomial likelihood, namely

$$\Pi_{ij}\hat{p}_{ij}^{n_{ij}}\Pi_i(1/\text{sqrt}(2\pi\sigma^2))\exp(-[\log(\hat{c}_i)-\log(c_i)]^2/(2\sigma^2)),$$

where the parameter to be estimated have been substituted by their estimates from SSPA,

$$\hat{c}_i = \Sigma_j \hat{c}_{ij},$$

and $\hat{p}_{ij} = \hat{c}_{ij}/\hat{c}_i$.
Here, the observations are

n_{ij} = the number of fish sampled in year i that were j yr-olds

and c_i = the catch in numbers in year i.

To find maximum likelihood estimates for SSPA parameters using the multinomial model, the quantity to be minimized is minus the log-likelihood:

$$-L = -\Sigma_{ij} n_i p_{ij} \log(\hat{p}_{ij}/p_{ij}) + \Sigma_i[\log(c_i) - \log(\hat{c}_i)]^2/(2\sigma^2) + \text{const.}$$

where the observed quantities are c_i, $n_i = \Sigma_j n_{ij}$, and $p_{ij} = n_{ij}/n_i$.

In the above expression $\log(\hat{p}_{ij})$ has been replaced with $\log(\hat{p}_{ij}/p_{ij})$, which is equivalent to subtracting a constant from the likelihood. This removes a "non-significant" portion of the likelihood and makes it easier to examine the model fit, and probably somewhat improves numerical performance. See Schnute and Fournier (1980) for a discussion of their "A" statistic. We shall call the model based on $-L$ the "multinomial maximum likelihood" model (MML). It can be noted that hypotheses concerning constraints on selectivities for the MML model can be tested by using a suitable likelihood ratio statistic.

Inclusion of Ageing Error

Fournier and Archibald (1982) and Methot (1986) propose incorporating ageing error into the model. Suppose the age determination process used in generating the observed data contains error described by the matrix:

$e_{j'j}$ = the probability that a fish of true age j' would be aged as j.

For the SSPA model this information can be incorporated by transforming p_{ij} to

$$\hat{a}_{ij} = \Sigma_{j'} \hat{p}_{ij'} e_{j'j}.$$

For the MML model all that is needed to incorporate ageing error into the model is to treat $\hat{a}_{ij}$ in the likelihood as if it were $\hat{p}_{ij}$.

Incorporating ageing error into the NLS model is equally simple. Simply estimate c_{ij} as $\hat{d}_{ij} = c_i \hat{a}_{ij}$ and treat $\hat{d}_{ij}$ as if it were $\hat{c}_{ij}$ in the least squares estimation.

Length-Based SSPA

Separable sequential population analysis based on lengths rather than ages can be derived as easily as incorporating ageing error into the model. This is because lengths may be thought of as just another set of ages with error (Methot and Hightower 1988). To accomplish this, assume we know the length-at-age matrix:

$\ell_{j'k}$ = the probability that a fish sampled of age j' will be of length k.

Since the annual samplings of length distributions (f_{ik}) are not themselves affected by ageing error, the fitting criterion given below can be used whether ageing error exists or not. However, when ageing error exists, the length-at-age matrix should somehow be adjusted for this error. The annual length distributions would then be estimated as

$$\hat{f}_{ik} = \Sigma_{j'} \hat{p}_{ij'} \ell_{j'k},$$

and the function to be minimized for estimating SSPA parameters under the MML model would be:

$$-L = \Sigma_{ik} n_i f_{ik} \log(\hat{f}_{ik}/f_{ik}) + \Sigma_i[\log(c_i) - \log(\hat{c}_i)]^2 / (2\sigma^2) + \text{const.}$$

For the NLS model, we can estimate the catch in numbers at length as $\hat{c}_{ik} = c_i \hat{f}_{ik}$. If the annual observations are the catch in numbers at length, c_{ik}, then the sum of squares to be minimized for estimating SSPA parameters is:

$$\text{SSC} = \Sigma_{ik}(\log(c_{ik}) - \log(\hat{c}_{ik}))^2.$$

Tuning the Models

In this paper I tune the models to independent survey estimates of fishable biomass $\{S^f_t\}$, where f denotes "fishable" and t is an index describing the years in which survey estimates were available. The problem of tuning was considered in detail for the NLS model by Kimura (1989). In SSPA, total and fishable biomass in year i are estimated by:

$$\hat{B}^t_i = \Sigma_j N_{ij} w_j = \text{total biomass},$$
$$\hat{B}^f_i = \Sigma_j N_{ij} s_j w_j = \text{fishable biomass},$$

where w_j is the average weight at age j. The sum of squares to be minimized under the NLS model is:

$$\text{SST} = \text{SSC} + \text{SSB} = \Sigma_{ij}(\log(c_{ij}) - \log(\hat{c}_{ij}))^2 + \lambda \Sigma_t(\log(S^f_t) - \log(\hat{B}^f_t))^2.$$

Assuming independent normally distributed errors, if

$$\lambda = \text{var}[\log(c_{ij})]/\text{var}[\log(S^f_t)],$$

then estimates obtained from minimizing SST will be maximum likelihood estimates for the NLS model. For simplicity $\text{var}[\log(S^f_t)]$ is assumed to be the same for all of the surveys, but it would be easy to specify a different λ_t for each survey. Kimura (1989) noted that $\text{var}[\log(X)] \approx cv(X)^2$, where cv is the coefficient of variation. By estimating $\text{var}[\log(c_{ij})]$ with the residual mean square error, and having an independent estimate of $cv(S^f_t)$, λ can easily be estimated.

Similarly, tuning can be accomplished for the MML model where the quantity to be minimized is

$$-L/\bar{n} = -\Sigma_{ij}(n_i/\bar{n}) p_{ij} \log(\hat{p}_{ij}/p_{ij}) + \lambda_1 \Sigma_i[\log(c_i) - \log(\hat{c}_i)]^2 + \lambda_2 \Sigma_t(\log(S^f_t) - \log(\hat{B}^f_t))^2.$$

The theoretically correct values for the λ_i are

$$\lambda_1 = 1.0/(2\bar{n}\text{var}[\log(c_i)]) \text{ and}$$
$$\lambda_2 = 1.0/(2\bar{n}\text{var}[\log(S^f_t)]).$$

I divided the log-likelihood by the average of the n_i ($\bar{n}$) because the resulting quantity came nearer to unity which might increase computational accuracy.

The above approaches to tuning the NLS and MML models provide maximum likelihood estimates when the λ terms are assumed to be known. However, when variance terms are to be estimated from the model, the optimization function must contain the full likelihood involving these terms, and the λ terms would have to be modified accordingly.

Tuning can be accomplished in a similar manner for length-based SSPA.

Choosing Between the NLS and MML Models

There are theoretical and practical considerations in choosing between the NLS and MML models. The theoretical question is simply "What sampling distribution is more correct for catch-at-age data: lognormal or multinomial?" And the practical considerations are "how well can available software estimate parameters for the NLS and MML models and are these models robust to alternative sampling assumptions?"

Fournier and Archibald (1982) argued that the multinomial sampling distribution, assumed by the MML approach, is the more valid when sampling age distributions. In particular they point out the heteroscedasticity and correlation in the observed p_{ij}'s. Certainly the multinomial model is plausible, but its correctness is not beyond debate.

Fournier and Archibald (1982) and Methot (1986) suggest an ad hoc limitation of the age sample size parameter to $n_i = 400$, regardless of the actual sample size which is usually much higher. They argue that there is variability other than the multinomial in the age distribution samples. For example, consider the variability resulting from using an age-length key. This ad hoc sample size limitation was not used in the present study.

The normal error model assumed by the least squares approach, historically perhaps the most successful statistical model, is also deserving of consideration. The NLS model has an advantage in that it does not a priori assume a specific level of modeling error as does the multinomial. By this I mean the following: given a sample size n_i and the multinomial assumption, the error level in the observations are set by the multinomial distribution, say var $(p_{ij}) \approx P_{ij}(1 - p_{ij})/n_i$. The NLS model allows the modeling error to take any value, which can in fact be estimated from the mean square error. The variability assumption under NLS, assuming lognormal error, is that the $cv(c_{ij})$ is constant for all i,j.

The practical consideration in choosing between NLS and MML centers around the performance of the best optimizing software available to apply to each method. Generally this will be a Levenberg–Marquardt nonlinear least squares program for the NLS model, and a quasi-Newton function minimizer for the MML model. Available IMSL software was used on a Burroughs B7800 computer: ZXSSQ for the NLS model, and ZXMIN for the MML model. The key options used in these routines was "strict decent" for ZXSSQ, and setting the initial Hessian equal to the identity matrix for ZXMIN. These subroutines have been documented in more recent versions under the names RNLIN and UMINF (IMSL 1989).

Because the form of the Hessian is known for the least squares routine, but unknown for a general function minimizer (Press et al. 1986), parameter estimation for the NLS model will generally be much more rapid than for the MML model.

In the present study, both IMSL optimizing routines appeared to work well. Initially, for both optimizing routines, the algorithms were terminated when all parameter estimates agreed to NSIG = 3 digits on successive iterations. This worked fine for ZXSSQ which was used to estimate parameters for the NLS model. However, for ZXMIN which was used for the MML model, this appeared to be an overly stringent convergence criterion, with many fits to simulated datasets exceeding the maximum limit set for function evaluation calls (MAXFN = 5000) without convergence having occurred.

Because the multinomial likelihood appeared to have been substantially optimized in 3 min of cpu time (see Simulation 1, below), a 3 min cpu time limit was used to determine convergence. However, it was found that this was generally insufficient cpu time for adequate convergence to have occurred. In order to assure that some degree of convergence had occurred, it was decided to set NSIG = 6 and terminate each fit when MAXFN = 5000 was exceeded. This basically set a cpu time limit of 17 min for each fit.

In summary, it is not clear whether NLS or MML is theoretically superior. Computationally, the NLS model can be expected to find parameter estimates much more rapidly than the MML model. There remains a question concerning the accuracy of parameters estimated from ZXMIN when the routine is terminated using cpu time as the convergence criterion. However, the time limit was set quite large, so substantial convergence was almost surely to have occurred. This fact was verified from estimates of the number of significant digits in the solutions.

In the simulation study that follows, it will become apparent that the NLS and MML models are robust to the alternative model's sampling assumption. Therefore, for practical purposes, the models do not appear to provide significantly different results. In order to reduce the computer time required for the simulations, the MML optimization criterion was used only in Simulations 1, 2, and 3.

Simulating SSPA Models

The simulations presented in this paper are based on a fit of the NLS model to walleye pollock (*Theragra chalcogramma*) in the Eastern Bering Sea (Kimura 1989; constraint $s_5 = 1$, assumption $\lambda = 10$). Table 1 shows the parameter values that were used as a basis for all of the simulations. All estimation algorithms assumed age 5 yr fish were fully available (i.e. $s_5 = 1$).

Simulations were carried out using both the lognormal and multinomial catch at age distribution assumptions. The lognormal assumption is that the c_{ij} and S_t^f are distributed as lognormal random variates. Further details for simulating and fitting these data using the NLS model are given in Kimura (1989).

The multinomial distribution assumption is that the n_{ij} are distributed according to the multinomial distribution with probabilities p_{ij}. For the multinomial simulations, c_i and S_t^f are assumed to be distributed as lognormal random variates.

All simulations assumed that survey estimates of fishable biomass (S_t^f) were available in the first and last years. This was done because a previous study (Kimura 1989) showed that estimates of population biomass could be strongly biased when survey biomass estimates were available for only the last year, even when the underlying modeling assumptions were correct. Because model fits essentially pass through the tuning points (i.e. survey biomass estimates), model performance can be best observed between these points.

All that remains to be specified are $cv(c_{ij})$, $cv(c_{ik})$, and $cv(S_t^f)$ for the lognormal simulations; and $cv(S_t^f)$, $cv(c_i)$, and n_i for the multinomial simulations. Except where noted, $cv(c_{ij})$, $cv(c_{ik})$, and $cv(S_t^f)$ were fixed for the lognormal simulations at 0.1. This value was chosen mainly to avoid computational difficulties that greater variability might cause.

Except where noted, $cv(c_i)$ and $cv(S_t^f)$ were set for the multinomial simulations at 0.1, and n_i was set at 2000. The sample size of 2000 was chosen because it is a practical (meaning attainable) value and makes the $cv(c_{ij})$ estimated from fitting the NLS model to the multinomial data (≈ 0.15) similar to that used in the lognormal simulations (0.1).

The simulation program was designed to allow data to be generated according to either the lognormal or multinomial distribution assumption, then analyzed using either the NLS or MML model. Thus, it was possible to consider not only how well the NLS and MML models performed, but how sensitive they were to the alternative distribution assumption.

A brief note can clarify how lognormal data can be fit using the MML model and vice versa. The lognormal data consists

TABLE 1. Assumed parameter values on which the simulations are based. These include the total population numbers (in millions) for the first year and age in which a year-class appears (N_{1j}, N_{i2}) in the data; the selectivities at age (s_j); the annual instantaneous fishing mortality rate for the fully available age-class (F_j); and the average weight at age (w_j) for pollock (kg). The annual instantaneous natural mortality rate was assumed to be $M = 0.3$ at all ages.

$N[i=1, j=2, 9]$ = 0.39594E + 01, 0.92258E + 01, 0.11375E + 02, 0.42692E + 01, 0.37263E + 01, 0.64562E + 01, 0.58712E + 01, 0.27260E + 01

$N[i=2, 22, j=2]$ = 0.11887E + 02, 0.95048E + 01, 0.14894E + 02, 0.11904E + 02, 0.11466E + 02, 0.11899E + 02, 0.10694E + 02, 0.45859E + 01, 0.66534E + 01, 0.16241E + 02, 0.16143E + 02, 0.15356E + 02, 0.12174E + 02, 0.11690E + 02, 0.18621E + 02, 0.32533E + 02, 0.10862E + 02, 0.71681E + 01, 0.46194E + 01, 0.68510E + 01, 0.40373E + 01

$s[j=2, 9]$ = 0.16353E + 00, 0.56751E + 00, 0.98875E + 00, 0.10000E + 01, 0.71800E + 00, 0.34898E + 00, 0.33776E + 00, 0.24195E + 00

$F[i=1,22]$ = 0.11588E − 01, 0.92938E − 02, 0.14915E − 01, 0.26238E − 01, 0.47219E − 01, 0.33733E − 01, 0.61316E − 01, 0.13499E + 00, 0.10626E + 00, 0.47385E + 00, 0.45903E + 00, 0.26210E + 00, 0.25948E + 00, 0.26182E + 00, 0.20129E + 00, 0.26742E + 00, 0.17069E + 00, 0.89668E − 01, 0.59226E − 01, 0.75179E − 01, 0.11151E + 00, 0.22002E + 00

$w[j=2,9]$ = 0.10500E + 00, 0.25100E + 00, 0.40900E + 00, 0.56500E + 00, 0.65000E + 00, 0.79800E + 00, 0.89200E + 00, 0.94500E + 00

of c_{ij} that are lognormal random variates. These were transformed to $c_i = \Sigma_j c_{ij}$ and $p_{ij} = c_{ij}/c_i$ for analysis by the MML model, with $cv(c_i) = 0.1$ and $n_i = 2000$ used in the λ variables. Conversely, the multinomial simulated data consists of c_i that are lognormal random variates, and $p_{ij} = n_{ij}/n_i$, where the n_{ij} were simulated from the multinomial distribution. These data were transformed to $c_{ij} = c_i p_{ij}$ for analysis by the NLS model, with $cv(c_{ij}) = 0.1$ used in the λ variables.

Because the simulations are based on known SSPA parameter values, the "true" total and fishable biomass values are known. Therefore it is possible to calculate the mean square error (MSE) of total and fishable biomass for every year i (below k indexes the 50 individual simulation results):

$$\text{MSE}(\hat{B}_i^t) = \Sigma_k(\hat{B}_{ik}^t - B_i^t)^2/50,$$
$$\text{MSE}\ (\hat{B}_i^f) = \Sigma_k(\hat{B}_{ik}^f - B_i^f)^2/50,$$

In order to make the simulation results more intuitively meaningful, the MSE values were transformed to coefficients of error defined as: $ce(\hat{B}_i^t) = \text{sqrt}[\text{MSE}(\hat{B}_i^t)]/B_i^t$ and $ce(\hat{B}_i^f) = \text{sqrt}[\text{MSE}(\hat{B}_i^f)]/B_i^f$. Note that these coefficients of error measure both bias and variability since $\text{E(MSE)} = \text{bias}^2 + \text{variance}$.

A total of seven simulation experiments were carried out, each designed to examine a specific aspect of these models.

Simulation 1. The main purpose of this simulation is to show that the NLS and MML optimization criteria substantially optimize the alternative likelihood function, whether the catch data are distributed multinomially or lognormally. This was done by fitting each of the two data sets using both optimization criteria. This simulation is unlike the others in this paper in that the coefficient of error described above will not be used to describe the results. Instead, time series traces will be used where the X-variable is computer cpu time for the optimization and the Y-variable is either −log(lognormal likelihood) or −log(multinomial likelihood), ignoring portions of the likelihood that are constant. The graphs are fairly complicated because we can trace the −log(lognormal likelihood) using both NLS and MML optimization criteria, and similarly for the −log(multinomial likelihood). In other words, we can trace −log(lognormal likelihood) as generated by optimizing either the NLS or MML models. This particular simulation is not replicated, and the population sizes that are estimated in the models were initially set at values 50% larger than those generating the simulation, so that convergence could be observed in the graphs. Note that Simulations 2 and 3 show the similarity of the NLS and MML results using replicated simulations.

Simulation 2. This simulation is based directly on the SSPA parameters in Table 1 with lognormal error. NLS is the correct model for these data, and I compare the results from NLS with those from MML.

Simulation 3. This simulation is based directly on the SSPA parameters in Table 1, but this time with multinomial error. MML is the correct model for these data, and I compare the results from MML with those from NLS.

Simulation 4. This simulation is based on the parameters in Table 1, with lognormal error. However, the catch at age data are transformed with ageing error prior to the addition of lognormal error. The ageing error is assumed to be normally distributed with the same coefficient of variation at each age. The distributions are truncated at the youngest and oldest ages so that young fish are over-aged and old fish are under-aged. The data are fit with an NLS model that assumes there is no ageing error.

Simulation 5. This simulation is based on the parameters in Table 1, and attempts to explore the problem of estimating selectivities with a functional form. I use the two parameter function suggested by Deriso et al. (1985):

$$s(j) = j^{\alpha} \exp(-\beta j)/[\max_j j^{\alpha} \exp(-\beta j)].$$

This function can be simplified by noting that for the selectivities in Table 1, $s_5 = 1.0$ is the maximum value. By differ-

TABLE 2. Unweighted averages of length-at-age for pollock in the Eastern Bering Sea. Length at age was averaged over sex, area, and time of year.

Length (cm)	Age (yr) 2	3	4	5	6	7	8	9
17	0.00050	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
18	0.00169	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
19	0.00587	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
20	0.01631	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
21	0.02537	0.00006	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
22	0.03219	0.00013	0.00006	0.00000	0.00006	0.00000	0.00000	0.00000
23	0.05681	0.00031	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
24	0.05987	0.00275	0.00006	0.00000	0.00000	0.00000	0.00000	0.00000
25	0.06350	0.00650	0.00006	0.00000	0.00000	0.00000	0.00006	0.00000
26	0.10981	0.01250	0.00062	0.00000	0.00000	0.00000	0.00000	0.00000
27	0.08881	0.02620	0.00175	0.00000	0.00000	0.00000	0.00000	0.00012
28	0.09168	0.03707	0.00318	0.00006	0.00000	0.00000	0.00000	0.00050
29	0.07150	0.05164	0.00618	0.00012	0.00000	0.00000	0.00000	0.00000
30	0.06918	0.06421	0.01161	0.00094	0.00025	0.00000	0.00000	0.00000
31	0.07400	0.06996	0.01742	0.00231	0.00000	0.00000	0.00000	0.00000
32	0.06331	0.07571	0.02778	0.00531	0.00006	0.00000	0.00000	0.00000
33	0.05068	0.07321	0.04196	0.00750	0.00038	0.00000	0.00000	0.00000
34	0.03425	0.08046	0.05070	0.01799	0.00306	0.00000	0.00000	0.00000
35	0.03500	0.09365	0.07767	0.03017	0.00532	0.00019	0.00000	0.00000
36	0.01987	0.10209	0.06918	0.03680	0.00938	0.00188	0.00000	0.00000
37	0.01962	0.06771	0.08079	0.05591	0.01608	0.00025	0.00000	0.00000
38	0.00656	0.06358	0.07842	0.05160	0.02640	0.00181	0.00332	0.00000
39	0.00144	0.05564	0.08210	0.06491	0.02765	0.01126	0.00000	0.00000
40	0.00037	0.03857	0.08485	0.07103	0.04704	0.01076	0.00357	0.00000
41	0.00150	0.02951	0.08135	0.07634	0.05823	0.01045	0.00013	0.00037
42	0.00000	0.02501	0.06805	0.07972	0.07093	0.02127	0.01670	0.00200
43	0.00000	0.00913	0.05906	0.08209	0.06956	0.04166	0.00807	0.00300
44	0.00019	0.00569	0.04189	0.06603	0.06468	0.03622	0.03203	0.01037
45	0.00000	0.00413	0.03808	0.05223	0.06418	0.06518	0.03616	0.03692
46	0.00006	0.00363	0.02079	0.05716	0.07556	0.05479	0.06957	0.03711
47	0.00000	0.00038	0.01948	0.05354	0.07093	0.07093	0.07976	0.05729
48	0.00000	0.00044	0.01492	0.04048	0.06361	0.05498	0.07457	0.07185
49	0.00000	0.00006	0.00862	0.03911	0.06549	0.06956	0.06675	0.09409
50	0.00000	0.00000	0.00512	0.02468	0.05492	0.07506	0.06068	0.08934
51	0.00000	0.00006	0.00531	0.01799	0.04435	0.07950	0.07557	0.06291
52	0.00000	0.00000	0.00125	0.02230	0.03015	0.08438	0.06037	0.05254
53	0.00000	0.00000	0.00100	0.02524	0.04010	0.06937	0.06444	0.07135
54	0.00000	0.00000	0.00006	0.00725	0.03165	0.04103	0.04661	0.04967
55	0.00006	0.00000	0.00019	0.00394	0.01745	0.03409	0.05055	0.05017
56	0.00000	0.00000	0.00000	0.00256	0.01320	0.04704	0.04479	0.03118
57	0.00000	0.00000	0.00000	0.00244	0.00588	0.02790	0.04379	0.04761
58	0.00000	0.00000	0.00000	0.00081	0.00776	0.02271	0.03084	0.04555
59	0.00000	0.00000	0.00037	0.00069	0.00688	0.01364	0.02778	0.02949
60	0.00000	0.00000	0.00000	0.00025	0.00219	0.01182	0.01608	0.03255
61	0.00000	0.00000	0.00000	0.00012	0.00288	0.01195	0.01120	0.03392
62	0.00000	0.00000	0.00000	0.00006	0.00175	0.00676	0.01996	0.01837
63	0.00000	0.00000	0.00000	0.00031	0.00025	0.00413	0.00826	0.01087
64	0.00000	0.00000	0.00000	0.00000	0.00119	0.00970	0.00738	0.01275
65	0.00000	0.00000	0.00000	0.00000	0.00006	0.00275	0.00932	0.01312
66	0.00000	0.00000	0.00006	0.00000	0.00031	0.00206	0.01307	0.00987
67	0.00000	0.00000	0.00000	0.00000	0.00000	0.00269	0.00744	0.00662
68	0.00000	0.00000	0.00000	0.00000	0.00000	0.00081	0.00369	0.00406
69	0.00000	0.00000	0.00000	0.00000	0.00006	0.00025	0.00106	0.00525
70	0.00000	0.00000	0.00000	0.00000	0.00006	0.00000	0.00094	0.00462
71	0.00000	0.00000	0.00000	0.00000	0.00006	0.00069	0.00194	0.00144
72	0.00000	0.00000	0.00000	0.00000	0.00000	0.00050	0.00044	0.00056
73	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00313	0.00256

entiating $\log[s(j)]$ with respect to j, we can infer that $\alpha = 5\beta$. Therefore the function can be simplified to a single parameter:

$$s(j) = (j/5)^{5\beta} \exp(-(j-5)\beta).$$

Simulations are made fitting the above function where the function is correct (i.e. all selectivities are described exactly by the functional form) and also where the function is incorrect (i.e. all selectivities are described by the individual "Doubleday" selectivities given in Table 1). As an additional comparison, these simulation runs are compared with a "standard" run that estimates Doubleday selectivities. Each simulation assumed lognormal error and was fit using the NLS model.

Simulation 6. This simulation compares length-based and age-based SSPA. The length-at-age data used for both generating

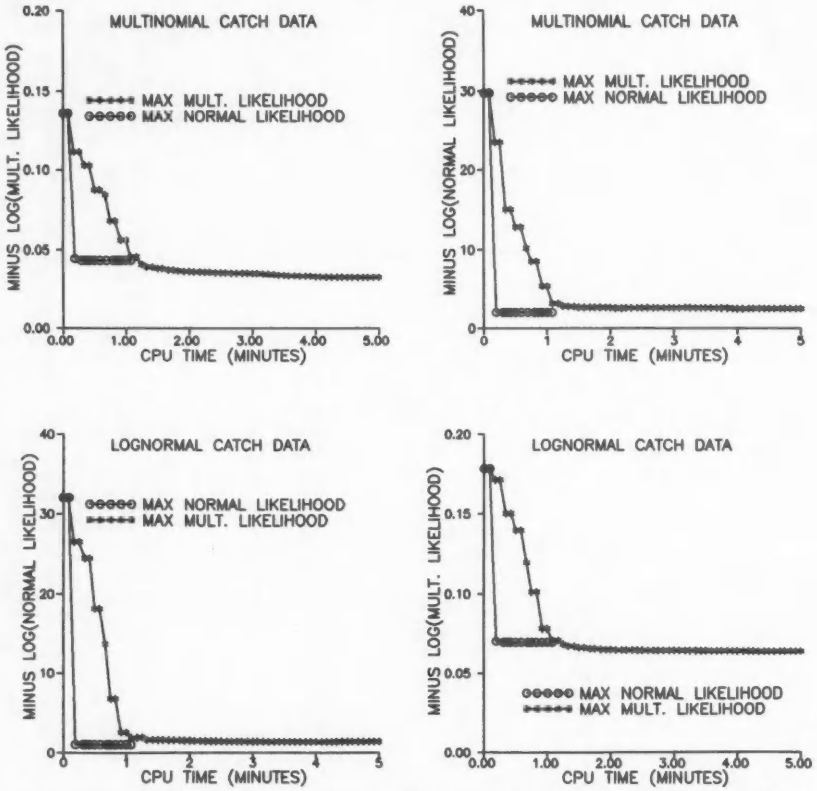

FIG. 1. Traces of $-\log$(likelihood) as a function of cpu time, when data are distributed multinomially (top) and lognormally (bottom). In each graph both NLS (max normal likelihood) and MML (max mult. likelihood) were used for optimization.

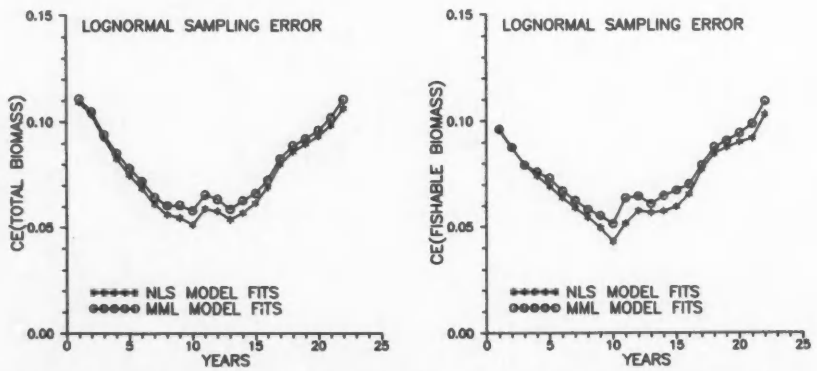

FIG. 2. Comparison of the coefficient of error of biomass estimates when data are fit using the NLS and MML models and catch-at-age are distributed with lognormal error.

and fitting the data can be found in Table 2. It was assumed that catch-at-age and catch-at-length were both distributed with a coefficient of variation of 0.1.

Simulation 7. This simulation examines how sample size affects model performance. For multinomial sample sizes of 500, 1000, 2000, and 3000 age observations per year, simulations were run using the NLS model to fit the data. In all simulations c_i was assumed to have a lognormal distribution with $cv(c_i) = 0.1$. In addition to the estimates of the coefficients of error of total and fishable biomass used to describe the results in the other simulations, I plot the square root of the residual mean square error vs. sample size. Because this value is roughly the

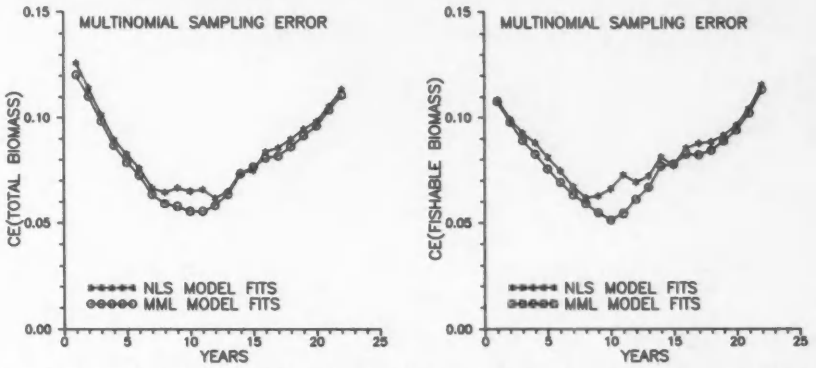

FIG. 3. Comparison of the coefficient of error of biomass estimates when data are fit using the NLS and MML models and catch-at-age samples are distributed according to the multinomial distribution.

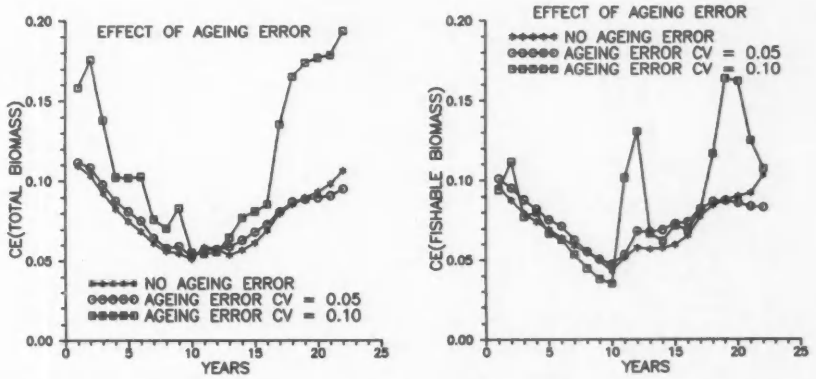

FIG. 4. Comparison of the coefficient of error of biomass estimates when data are fit using the NLS model and catch-at-age contain ageing error prior to the addition of lognormal error.

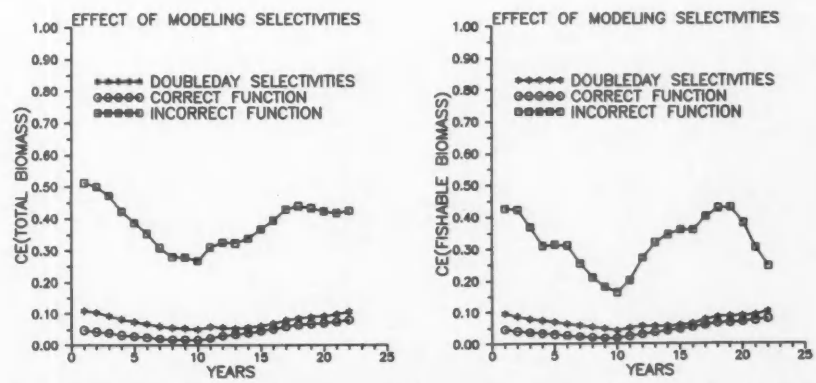

FIG. 5. Comparison of the coefficient of error of biomass estimates when data are fit using the NLS model with a functional form for selectivities, and catches at age are distributed with lognormal error. Models are fit assuming a correct function specification, and incorrect specification, and a baseline result which fits the Doubleday selectivities.

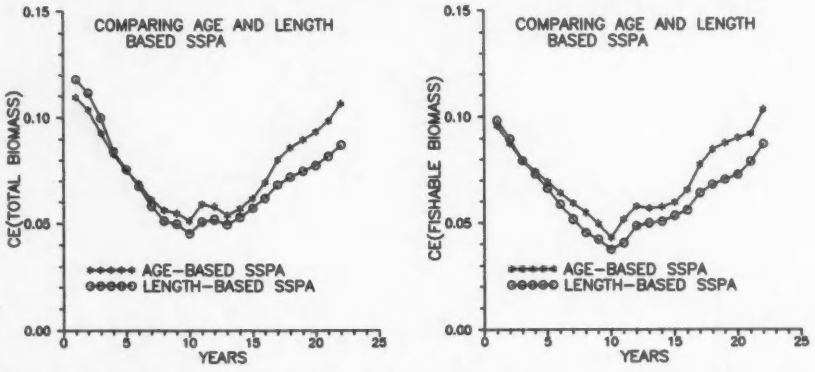

FIG. 6. Comparison of the coefficient of error of biomass estimates when the NLS model is fit to simulations of catch at length and catch at age data containing lognormal error.

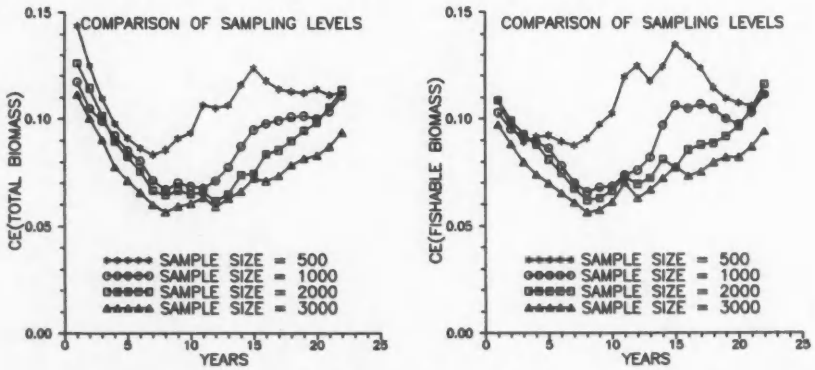

FIG. 7. Comparison of the coefficient of error of biomass estimates when data are fit using the NLS model and catch at age samples vary in size and are distributed according to the multinomial distribution.

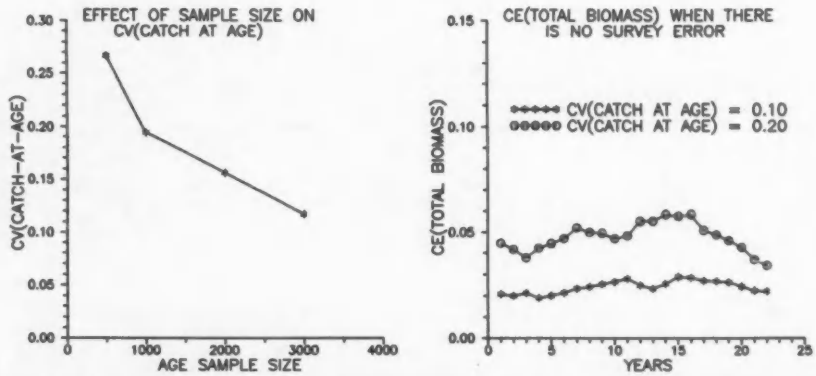
EFFECT OF SAMPLE SIZE ON CV(CATCH AT AGE)
CV(CATCH-AT-AGE)
AGE SAMPLE SIZE
CE(TOTAL BIOMASS) WHEN THERE IS NO SURVEY ERROR
CV(CATCH AT AGE) = 0.10
CV(CATCH AT AGE) = 0.20
CE(TOTAL BIOMASS)
YEARS

FIG. 8. Left: An approximate average coefficient of variation of catch at age, as a function of sample size, estimated from the same simulation runs used to generate Fig. 7. Right: The coefficient of variation of biomass estimates when catch at age are distributed with lognormal error and data are fit using the NLS model. The model is tuned to the errorless "true" fishable biomass in the first and last year.

TABLE 3. A comparison of Doubleday selectivities used in the simulations (Table 1) with values estimated by fitting a simplified version of the selectivity curve suggested by Deriso et al (1985). Selectivities were estimated by fitting an NLS model, including the selectivity curve, to data containing essentially zero sampling variability.

Age	Doubleday selectivities	Estimated selectivities
2	0.164	0.167
3	0.568	0.534
4	0.989	0.877
5	1.000	1.000
6	0.718	0.905
7	0.349	0.698
8	0.338	0.479
9	0.242	0.301

$cv(c_{ij})$, I also examine the cv of estimated total biomass when $cv(c_{ij}) = 0.1$ and 0.2 and there is no error in the survey estimates of fishable biomass.

Results from the Simulations

Results from a simulation with a single replication is given in Fig. 1, and simulations with fifty replications per simulation given in Fig. 2–8. Generally with 50 replications on a Burroughs B7800, an NLS simulation took 0.67 hr of cpu time, and an MML simulation took 14.0 hr of cpu time. The cpu time limit per simulation that was used often carried MML model fits beyond the single precision limit (11 digits of accuracy) and computations were terminated. However, estimates of the number of significant digits in the solutions indicated that results were meaningful.

Most of the graphs of *ce* (biomass) appear as strings hung at a value 0.1 at each end. This is because the ends were tuned to survey estimates of fishable biomass that were assumed to have cv's of 0.1. The relative position of graphs within a figure, and not the absolute position of a graph, should be the main item of interest.

All of the simulation experiments appeared to give fairly clear results:

Simulation 1. Fig. 1 illustrates several phenomena. Most obvious is how much more rapid NLS optimization is compared with MML optimization. Next, is how both methods of optimization at least roughly maximize the alternative likelihood (i.e. NLS roughly maximizes the lognormal likelihood). Finally, note how the MML optimization slows dramatically following rapid initial progress. The parameter estimates from MML optimization are not generally satisfactory after 5 min of cpu time. It is safer to overdo than underdo the amount of computation.

Simulation 2. Fig. 2 indicates that when catch-at-age data are lognormally distributed, the MML model gives nearly the same performance as the theoretically correct NLS model. Thus, the MML model apears robust to lognormal catch-at-age data.

Simulation 3. Fig. 3 indicates that when catch-at-age data are multinomially distributed, the NLS model gives nearly the same performance as the theoretically correct MML model. Although a little increase in mean square error is evident in the middle years (Fig. 3), the NLS model appears to be fairly robust to multinomial catch-at-age data.

Simulation 4. Fig. 4 indicates that when ageing error is random, and of moderate magnitude ($cv = 0.05$), the modeling error may not be affected. Typically, between-reader ageing error is of this magnitude (D. K. Kimura and J. J. Lyons, unpubl. data). However, if ageing error is higher ($cv = 0.10$), modeling error may be dramatically increased (Fig. 4).

Simulation 5. Fig. 5 shows that substantial error reduction is possible when a "correct" selectivity function is used in fitting the SSPA model. However, if there is moderate lack-of-fit between the true selectivities and the selectivity function (Table 3), then errors in estimating biomass can dramatically increase (Fig. 5).

Simulation 6. Fig. 6 illustrates that length-based SSPA may be feasible for some species where the distribution of length-at-age is fairly constant from year to year.

Simulation 7. Fig. 7 indicates that if sampling of ages follows the multinomial model, then a sample size of 1000 ages may be sufficient for performing SSPA. To further explore this, the $cv(c_{ij})$ estimated from the sqrt (average MSE) from the NLS model fit was plotted against sample size (Fig. 8, left). This figure shows that for a sample size of 1000 ages, $cv(c_{ij}) \approx 0.20$. If we assume that the survey estimates used for tuning have no error, then the *ce* (total biomass) is small (Fig. 8, right). Therefore, a sample size of 1000 ages/yr, under multinomial sampling, should be sufficient.

Discussion

The purpose of this paper has been to show that SSPA models constitute a recognizable class with a wide range of applicability. Their flexibility in accepting a variety of assumptions makes them especially amenable to exploration through simulation.

I have presented a range of simulations in order to provide provisional answers to some likely questions regarding these models. The answers may depend heavily on the population being modeled and the constraints that are employed, so readers are encouraged to perform their own simulations customized to their populations and problems of interest.

Regarding the simulations performed here, we conclude that that the NLS and MML versions of SSPA are mutually robust (i.e. they are robust to the alternative modeling assumption) and are for practical purposes the same model. However, the NLS method of parameter estimation appears much more rapid.

Low levels of ageing error did not increase error in modeling results; however, higher levels of ageing error were found to be damaging. Therefore, when high levels of ageing error are known to exist, the modeling should somehow try to take this into account (see e.g. Methot and Hightower 1988; or Deriso et al. 1989).

The SSPA model appears to be sensitive to assumptions regarding selectivities. In an earlier paper (Kimura 1989) I found that the NLS model gave extremely biased results when fixed (almost knife-edged) selectivities were incorrectly assumed. Similarly, in Simulation 5, when an incorrect functional form was assumed for the selectivity curve biomass estimates showed greatly increased error.

Simulation with length-based SSPA was encouraging. Length-based SSPA may be most useful in cases where direct ageing is difficult, where inventories of length distributions without ages are available, when growth continues throughout a fish's life span, and when length-at-age distributions are roughly the same over all years.

The final simulation regarding the effect of age sample size on the variability of modeling results indicates that if the sampling problem can be reduced to one of pure multinomial sampling, the amount of age data required in the annual sampling of fisheries catches is remarkably modest, perhaps around 1000 observations. One reviewer pointed out that this simulation should be deleted because it would result in grossly incorrect sampling recommendations, since sampling is never multinomial. Although I agree these results should not be used to set sampling levels, they, however, point out once again that the main problem of sampling is usually the representativeness of the samples, and not the sample size. This brings us to our final disclaimer.

Because of the unrealistic nature of the assumptions required when modeling, the conclusions here do not represent "truth" in the scientific sense, but rather information concerning how these models work. They give an indication of the meaningfulness of the results when using these models, and where the points of modeling sensitivity might be. The frustrating and interesting thing about modeling is that what is an acceptable assumption to one modeler in a particular context may appear foolhardy to another. For example, distribution assumptions will seldom be met, ageing error is hardly ever random, it is unlikely true selectivities follow a nice functional form, and sampling of fisheries will never be multinomial. Nevertheless, if we are to use these models we should understand how these models work and simulation is one important way to help us find out.

Acknowledgements

I thank D. A. Fournier, J. M. Hoenig, R. D. Methot, and G. G. Thompson for some very helpful reviews.

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A Method for Forecasting the Timing of Ripeness in Pacific Herring (*Clupea harengus pallasi*) Females

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Ware, D. M., and R. W. Tanasichuk. 1990. A method for forecasting the timing of ripeness in Pacific herring (*Clupea harengus pallasi*) females. Can. J. Fish. Aquat. Sci. 47: 2375–2379.

A procedure was developed to forecast when Pacific herring females become ripe. The method uses a size-independent quantitative measure of maturity (*D*), which is the difference between the observed GSI (gonosomatic index) and the GSI expected at full maturity (*Q*) for a given body weight. We use *D* to segregate fish within samples, and then to split the samples into maturity groups. These groups often reflect discrete spawning waves. We regress mean *D* values for samples within a maturity group against date of collection to calculate when the observed GSI will equal *Q*, that is, when those females will be ripe. Analysis of data for 14 maturity groups indicates that the date of ripeness can be forecast with an average accuracy of 0.5 d, 5–6 d before it occurs. This procedure should be useful to fishery managers concerned with staging fisheries on mature fish and to investigators who need to know when a spawning stock reaches full maturity.

Une méthode permettant de prévoir le moment auquel les femelles de hareng du Pacifique sont pleines a été mise au point. Cette méthode est fondée sur une mesure quantitative de la maturité indépendante de la taille (*D*) correspondant à la différence entre l'indice gonosomatique observé et celui prévu à pleine maturité (Q) pour un poids corporel donné. Le paramètre D est utilisé pour séparer les poissons à l'intérieur des échantillons, puis pour diviser ces échantillons en groupes fondés sur le degré de maturité des poissons. Ces groupes correspondent souvent à différentes cohortes de fraie. Nous faisons une régression entre les valeurs moyennes de *D* pour les échantillons d'un groupe de maturité et la date de récolte des poissons pour calculer le moment où l'indice gonosomatique observé sera égal à *Q*, c'est-à-dire le moment où les femelles seront pleines. Les analyses des données de 14 groupes de maturité montrent que cette date peut être prévue 5–6 j d'avance avec une précision moyenne de 0,5 j. Cette méthode devrait s'avérer utile aux gestionnaires veillant à ce que les poissons soient pêchés au bon moment et aux chercheurs qui doivent connaître le moment où les stocks reproducteurs arrivent à maturité.

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Pacific herring is the most valuable commercial fish species on the British Columbia coast because of the lucrative roe fishery. Between 1982 and 1987, roe herring accounted for about 25% of the average annual landed value ($200 000 000) of all commercial fin-and shellfish species taken in British Columbia waters (P. Macgillivary, Economic and Commercial Analysis Division, Fisheries and Oceans Canada, Vancouver, B.C., pers. comm.). Prices to fishermen averaged $2,200 and 2,800·ton^{-1} for seined and gillnetted fish, respectively. This translated into an average annual income of $140 000 per seiner and $25 000 per gill-netter. These incomes become more impressive after considering that seine fisheries usually last about an hour, and a gillnet season about a day.

The highest prices are paid for top quality (ripe) roe and drop off sharply with a decline in quality. Consequently, managers of seine roe fisheries walk an incredible tight-rope every season. If the fishery occurs too early, then roe quality and prices will be low. Conversely, fishing opportunities could be lost if a manager waits too long and spawning begins. Gillnet fisheries are not at risk because herring are intercepted as they move to spawning beaches. However, there are still problems associated with uncertainty in the timing and location of gillnet fisheries.

Herring fishery managers now depend on a qualitative measure of maturity (roe yield) to determine when to stage a roe fishery. The roe yield reflects the proportion, by weight, of ripe roe in the catch. Unfortunately, it does not allow managers to anticipate a fishery. Females can progress from maturity stage V (Hay 1985) to ripeness (stage VI) within 24 h. In addition, different maturity groups of fish can co-occur and therefore complicate roe yield trends. Within this web of uncertainty, fishery managers must also speculate what the maximum roe yield will be.

Our study had two objectives: (1) to devise a simple method to estimate when female herring will ripen and (2) to develop an equation to estimate the maximum potential roe yield. Originally, we hoped to develop a procedure for forecasting the date of spawning. However, Ware and Tanasichuk (1989) found that female herring can, especially in locations where the tidal cycle influences spawning time (e.g. Barkley Sound, lower east coast Queen Charlotte Islands), hold at full maturity for a week or more before spawning. Consequently, our procedure is stock-specific since it forecasts spawning time for stocks which spawn as soon as the females ripen, and predicts the time of ripening (stage VI) for stocks influenced by the tides.

Materials and Methods

The forecasting method uses samples of fresh herring collected by purse seiners. The Canadian Department of Fisheries

and Oceans routinely charters seiners over the last month before spawning. The objectives of these charters are to locate aggregations of maturing fish, provide in-season biomass estimates, collect biological samples, and monitor roe yield. Our analysis used samples from sets made in the Strait of Georgia during 1983–85, the Prince Rupert area (Port Simpson/Big Bay, Kitkatla) in 1984, along the lower east coast of the Queen Charlotte Islands in 1986, and in Barkley Sound during 1987–89 (Fig. 1).

Fifty females from each set were sampled, either on board seiners or, when convenient, at the Pacific Biological Station. Individual total body and ovary weights were measured to the nearest 0.1 g, and the gonosomatic index (GSI = ovarian weight*100/total body weight) was calculated. This equation was used to define the GSI because variations in body condition factor in Pacific herring affect ovary weight. Since the GSI is more highly correlated with body weight than it is with length, we obtained a much better forecast of when herring will ripen by expressing the GSI as a function of body weight.

The date when female herring ripened was estimated from information provided by roe yield tests (e.g. Armstrong 1985). These tests indicate the proportion of females in roe grades 3 (maturity stage IV), 2 (maturity stage V), and 1 (maturity stage VI). Females were considered ripe when the proportion in maturity stage VI was maximal, and above 90%.

The Original Procedure

The method evolved from a preliminary examination of the variation in GSI over the last few weeks before spawning in Barkley Sound in 1982 (D. M. Ware, unpubl. data). The gonosomatic index appeared to increase linearly with time to 26.5%, after which spawning began.

In-season forecasting work began in the Strait of Georgia in 1983. Forecasts were made by regressing mean GSI of each sample against date of collection and then calculating when the mean GSI would reach 26.5%. It soon became clear, however, that not all female herring were ripe at a GSI of 26.5%. In addition, it was sometimes difficult to separate different maturity groups of fish. Different maturity groups can co-occur when fish from different spawning populations, or different spawning waves of the same population, mix.

Estimating GSI at Full Maturity (Q)

To improve the method, we derived an equation to accurately estimate the GSI at full maturity (Q). The equation had to con-

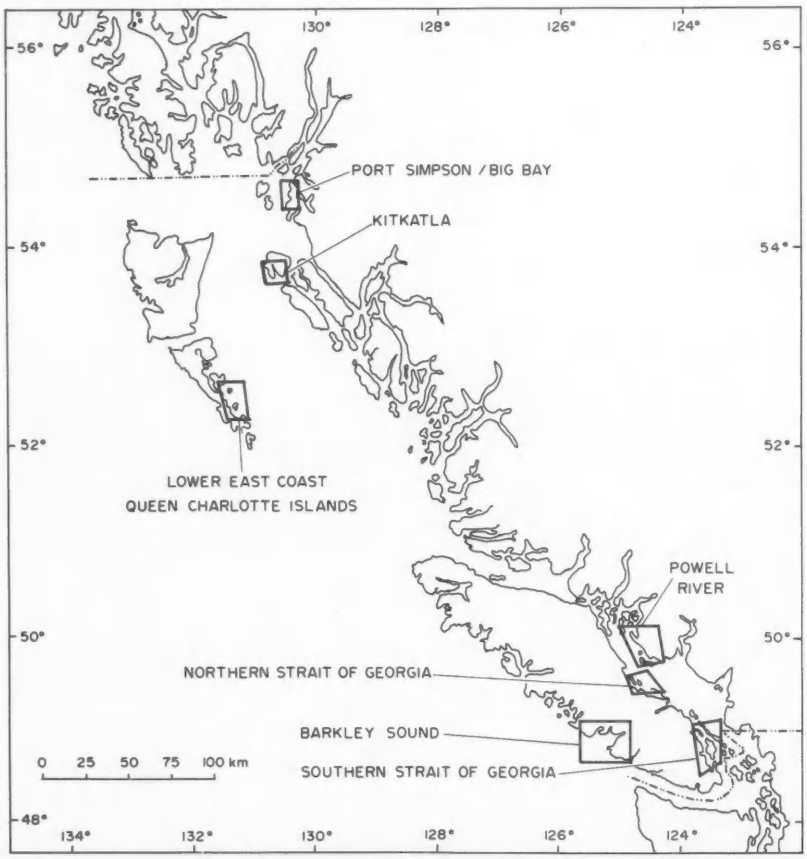

FIG. 1. Sampling locations: Strait of Georgia (Area 14N (Northern Strait)), Area 15 (Powell River), Area 17S (Southern Strait), Barkley Sound (Area 23), Kitkatla (Area 5), Port Simpson/Big Bay (Area 4), lower east coast Queen Charlotte Islands (Area 2E).

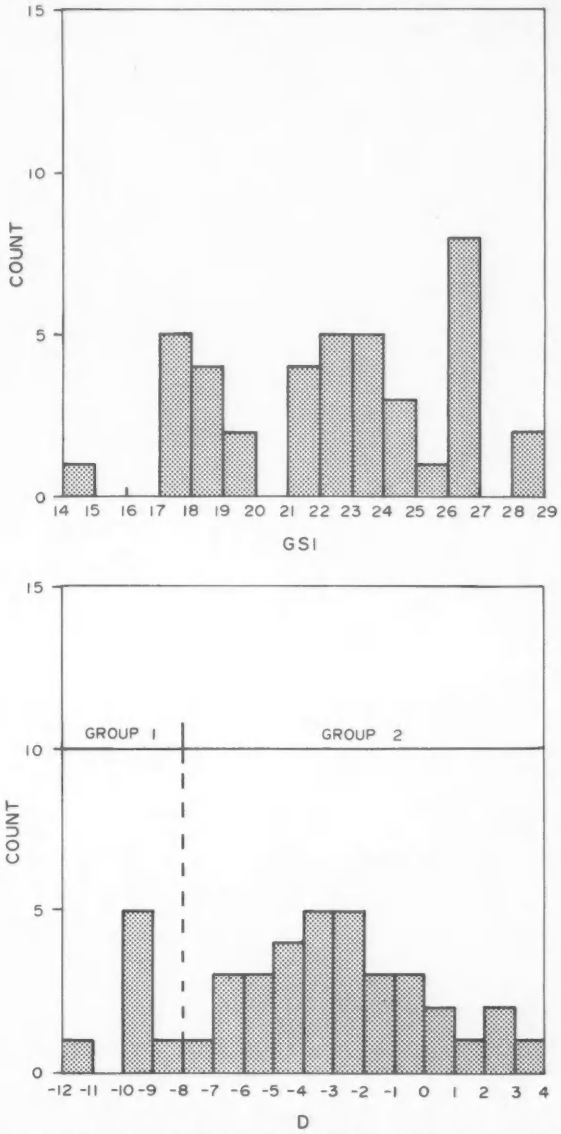

FIG. 2. Histograms of GSI (Fig. 2a) and D (Fig. 2b) for a sample of herring collected in Barkley Sound, 1989. Note that the maturity groups are obvious only in the distribution of D.

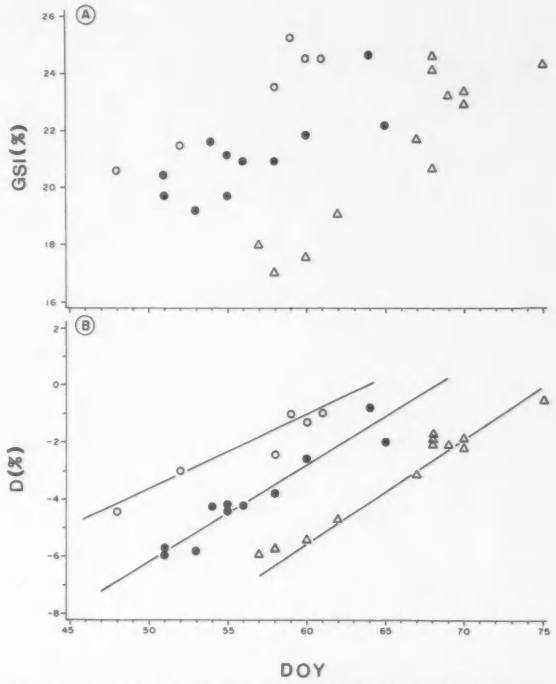

FIG. 3. Plots of mean GSI (Fig. 3a) and D (Fig. 3b) against sampling date for herring spawning in the northern Strait of Georgia, 1984. Fig. 3b shows that three maturity groups (represented by the different symbols) occur; the segregation of the groups is less obvious in Fig. 3a. Unlike GSI, D conveys a sense of how close fish are to being fully mature. The lines in Fig. 3b are fitted regressions, for each maturity group.

sider the effect of fish size and freezing. Data for thawed fish, obtained from the routine biological sampling program, showed that Q was an increasing function of female weight (Ware and Tanasichuk 1989). However, measurements indicated that size-specific Q for fish which had been frozen was about 3% higher than it was for fresh fish (R. W. Tanasichuk, unpubl. data). Consequently, we calculated a preliminary equation for estimating Q from fresh body weight (W, g) ($Q = 6.08*W^{0.28}$) based on the relationship between ripe ovary weight (R_o) and fresh body weight ($R_o = 0.06*W^{1.28}$, $n = 124$, $r = 0.98$, $P < 0.0001$) for the only available data; specifically, from fish collected in the northern Strait of Georgia in 1984. The resulting estimates of Q were too low and calculated dates of ripeness proved to be too early. We revised the equation ($Q = 7.28*W^{0.26}$) by using the mean slope and intercept of the fresh and frozen Q-total body weight regressions. This equation was used to "hindcast" the date of ripeness for herring in the database, and was field-tested by forecasting the timing of ripeness for Barkley Sound herring in 1988 and 1989.

The Modified Procedure

The current procedure uses the difference between Q and the observed mean GSI (D) as the measure of maturity. Initially, frequency histograms of the calculated variable D are used to assign fish within a sample to their respective maturity groups (Fig. 2). The average D for each maturity group within a sample is then used to segregate (sub)samples of fish into their respective maturity groups (Fig. 3). We then regress mean D against sampling date to estimate when $D = 0$, that is, when the average GSI of a maturity group of fish equals the spawning GSI for its average body weight. Operationally, we begin forecasting with a minimum of 5 d of data and when the regressions become statistically significant ($P < 0.05$). The forecasts are updated with additional sampling.

Results and Discussion

Table 1 shows how accurately our method was able to hindcast the timing of ripeness for 14 maturity groups of herring

TABLE 1. Forecasted dates of ripeness by area and maturity group (dd/mm, first row). The dates successive forecasts were made are listed in the second row. The date of ripeness is given in parentheses after the spawning date in instances where females held at full maturity. The forecast used to determine the accuracy of the method (diff) in each case is underlined. Diff = forecast date — observed date of ripeness. U = unknown roe yield.

Year	Maturity group	Forecast number 1	2	3	4	5	6	7	8	Dates (dd/mm) spawning began	Diff	Maximum roe yield (%) Predicted	Observed
						Northern Strait of Georgia (Area 14N)							
1983	1	27/02								28/02	−1	12.8	U
		25/02											
1984	1	06/03	03/03							03/03	0	12.8	U
		29/02	01/03										
	2	08/03	07/03	06/03	08/03					08/03	0	12.6	U
		27/02	29/02	04/03	05/03								
	3	17/03	14/03	13/03	15/03	15/03				18/03	−3	12.3	U
		07/03	08/03	09/03	10/03	15/03							
1985	1	08/03								07/03	+1	12.4	13.2
		04/03											
	2	09/03	09/03							07/03	+2	11.9	U
		04/03	05/03										
						Powell River (Area 15)							
1983	1	15/03	17/03	11/03						11/03	0	12.4	U
		01/03	03/03	06/03									
1984	1	08/03	09/03	07/03						08/03	0	12.2	U
		01/03	02/03	03/03									
	2	11/03								12/03	−1	12.2	U
		04/03											
1985	1	10/03	07/03							06/03	+1	11.8	U
		02/03	03/03										
						Southern Strait of Georgia (Area 17S)							
1984	1	12/03	12/03	13/03	13/03	13/03	14/03	15/03	13/03	16/03	−3	12.5	U
		01/03	03/03	05/03	06/03	07/03	08/03	09/03	11/03				
						Lower east coast Queen Charlotte Islands (Area 2E)							
1986	1	26/03	21/03	23/03						06/04(23/03)	−2	14.2	14.2
		16/03	17/03	18/03									
						Port Simpson/Big Bay, Kitkatla (Areas 4 and 5)							
1984	1	26/03	31/03	26/03						26/03	0	12.5	U
		19/03	20/03	21/03									
						Barkley Sound (Area 23)							
1987	1	07/03	06/03	08/03	05/03	09/03	09/03	09/03	10/03	19/03(11/03)	−2	13.2	13.1
		27/02	28/02	01/03	02/03	05/03	06/03	07/03	08/03				
						Barkley Sound Field Trials							
1988	1	16/03	15/03	13/03	13/03	11/03	12/03	12/03		19/03(11/03)	0	12.6	12.4
		02/03	05/03	07/03	08/03	09/03	10/03	11/03					
1989	1	14/03	08/03	09/03	10/03	10/03	10/03	10/03	10/03	18/03(12/03)	−2	13.1	13.3
		03/03	04/03	05/03	06/03	07/03	08/03	09/03	10/03				

sample between 1983 and 1987. To determine the accuracy of the method we selected the first forecast in each series which was within 3 d of the observed date of spawning (or ripeness in two instances where females held at full maturity); the second criterion was that all succeeding forecasts also had to be within 3 d of the observed date of spawning. For example, in the northern Strait of Georgia in 1984, we could have forecast on February 27 that maturity group 2 would spawn on March 8; the succeeding three forecasts produced a similar date. This group of fish was observed to spawn on March 8; so, in this case, we were able to forecast the exact date of spawning (Difference = forecast date — observed date of spawning = 0) 10 d before the event. With respect to the entire set of 14 cases, spawning (or ripeness) was "forecast" with an average accuracy of −0.5 d, 5.7 d in advance; the minus sign indicates that the forecast lead the observed date, on average, by 0.5 d.

The results of two in-season field tests of the procedure in Barkley Sound (1988 and 1989) are shown at the bottom of Table 1. In these trials we were able to forecast the time of ripeness with an average accuracy of −1 d, 4 d in advance. These forecasts proved to be useful in helping the fisheries manager decide when to stage the seine fisheries.

The maximum roe yield that can be obtained from a given herring maturity group is simply equal to $Q/2$, assuming a 50:50 sex ratio. A paired t-test of the predicted and observed roe yields listed in Table 1, indicates that our predictions of roe yield were also accurate ($t = 0.48$, $0.9 < P < 0.5$). Thus our forecasting method appears to be able to provide accurate forecasts of when herring will spawn, and the expected roe yield of the fishery.

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Influence of Four Species of Helminth Parasites on Orientation of Seaward Migrating Sockeye Salmon (*Oncorhynchus nerka*) Smolts

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Each of four helminth parasite species, including the cestodes *Eubothrium salvelini*, *Diphyllobothrium dendriticum*, and *Proteocephalus* sp. and the nematode *Philonema oncorhynchi*, was found to influence the orientation of seaward-migrating sockeye salmon (*Oncorhynchus nerka*) smolts from the Great Central Lake area, Vancouver Island, British Columbia. Smolts placed in circular tanks were allowed to exit into evenly spaced bucket traps, and fish from five such runs were measured, sexed, and examined for the presence of parasites. Fish were categorized on the basis of infection status ("+" vs. "−") for each of the four parasite species found. The +/− groups did not differ for any of the parasite categories with respect to either bucket distributions or overall orientation directions. However, "−" groups did exhibit consistently greater departure from random distribution than their "+" counterparts. This finding suggests interference of parasites with smolt orientation, which could in turn have important implications for smolt survival. This finding also suggests that parasite infection may account to some extent for the considerable variability observed in the migratory behaviour of smolts.

On a observé que quatre espèces de parasites, soit les cestodes *Eubothrium salvelini*, *Diphyllobothrium dendriticum* et *Proteocephalus* sp. et le nématode *Philonema oncorhynchi*, ont chacun une influence sur l'orientation des smolts de saumon rouge (*Oncorhynchus nerka*) de la région du lac Great Central, sur l'île Vancouver en Colombie-Britannique, lors de leur migration vers la mer. Les smolts ont été placés dans des réservoirs circulaires et pouvaient en sortir par une série d'ouvertures également espacées débouchant sur des récipients. Cinq groupes de smolts ont été soumis à cette expérience; ces poissons ont été mesurés, on en a déterminé le sexe et ils ont été examinés pour voir s'ils étaient parasités. Ils ont été classés suivant qu'ils étaient parasités ("+") ou non ("−"), et ce pour chacune des quatre espèces de parasites. Les groupes +/− n'ont pas montré de différence suivant la nature du parasite quant à leur distribution dans les récipients et aux directions empruntées de façon globale. Cependant, les groupes "−" ont montré un plus grande déviation par rapport à la distribution au hasard que leurs contreparties "+". Ce résultat laisse entendre que les parasites influeraient sur l'orientation des smolts, ce qui pourraient avoir des incidences importantes sur leur survie, et que le parasitisme pourrait, dans une certaine mesure, expliquer la variabilité importante observée dans le comportement migratoire des smolts.

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Migration plays an important role in the biology of many vertebrate and invertebrate species (Baker 1978; Able 1980; Dingle 1980). However, migration is dependent upon a complex interplay of environmental cues with sophisticated, carefully tuned, innate behavioural and physiological mechanisms (Baker 1978; Meier and Fivizzani 1980) and would, as such, appear to be highly vulnerable to adverse influence.

Parasites have been shown to exert a wide range of physiological and behavioural effects upon their hosts (Holmes and Bethel 1972; Holmes 1976, 1982; Moore 1984; Sindermann 1987; Godin and Sproul 1988). In some cases, adverse effects are advantageous to the parasite because they increase the likelihood of transmission (Godin and Sproul 1988), and these effects may include interference with host navigation and orientation. For example, the periodic mass strandings of live whales and dolphins remain unexplained, but Ridgway and Dailey (1972), Dailey and Walker (1978), McColl and Obendorf (1982), and Morimitsu et al. (1986) have all presented circumstantial evidence for the involvement of trematodes and other helminths in various dolphin stranding incidents. Curtis (1987) described trematode-caused beach stranding of estuarine snails (*Ilyanassa obsoleta*), and Flecker and Allan (1988: 41) found "a pronounced bias" toward upstream flight in nematode-parasitized female mayflies (*Baetis* spp.). Thus, parasites clearly have the potential to affect animal navigation and orientation, but, to date, there have otherwise been no experimental studies focusing upon parasite interference with migration-related behaviour.

The extraordinary migratory capabilities of sockeye salmon (*Oncorhynchus nerka*) make these fish excellent subjects for investigating the influence of parasitism upon orientation. Sockeye usually spend the first 1 or 2 yr of their life in a lake before reaching the "smolt" stage, at which point they migrate through rivers, estuaries, and inlets to the sea, where they journey thousands of kilometres over the course of 2 or 3 yr before returning to their natal stream to spawn (Neave 1964; Groot 1965; Hartman et al. 1967; Brannon et al. 1981; Margolis 1982; Quinn et al. 1987). In large lakes, smolts undertake migrations of up to 180 km to reach the lake's outlet (Groot 1972), and,

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depending upon their location, different demes within a lake may follow markedly different routes to that outlet. Nevertheless, Groot (1965, 1972) found the migrations of such demes to be the result of clearly oriented movement in directions appropriate to the shortest exit routes. Moreover, the mechanisms guiding these movements are sophisticated, innate systems involving magnetic and celestial cues (Groot 1965, 1972, 1981; Quinn 1980, 1981; Brannon et al. 1981; Quinn and Brannon 1982).

This paper presents results of an experimental investigation of the influence of four species of helminth parasites on the orientation of seaward migrating sockeye salmon smolts under experimental conditions.

Materials and Methods

Orientation Experiment

The present study was conducted in conjunction with research carried out by Dr. C. Groot (Pacific Biological Station, Nanaimo, British Columbia, Canada, V9R 5K6) on orientation of seaward migrating sockeye salmon smolts leaving Great Central Lake via Robertson Creek on Vancouver Island, British Columbia (see also Quinn and Brannon (1982) and Quinn and Groot (1989). Groot conducted these experiments at the Robertson Creek hatchery in circular, 1.8 m diameter fiberglass tanks open to the sky. A water depth of 30 cm was maintained by water pumped from Robertson Creek into the center of each tank and drained via eight, 32 mm diameter round holes spaced evenly around the wall of the tank. Water levels in the tanks were maintained at the centers of the outlet holes, and buckets placed underneath each outflow caught fish swimming out through these holes. Bucket 1 corresponded to a direction of geographic north (0°), and buckets 2–8 corresponded to +45° increments in compass bearing. The use of two such tanks (referred to as "tank 1" and "tank 2") made possible two runs per night.

Experimental runs were begun at approximately 10:30, at which time between 300 and 600 outmigrating smolts trapped in Robertson Creek the preceding night were placed in each tank. Fish were counted only after each run in order to minimize the handling of smolts prior to experimental treatment. The fish immediately swam to the bottom upon placement in the tank, where they remained until dusk fell at approximately 21:00. At that time, fish moved toward the surface, and, during the night, some swam to the outlets. This diel behavioural pattern is consonant with field observations of migrating smolts, which have been found to swim near the surface between dusk and dawn and spend daylight hours at greater depths (Hartman et al. 1967; Groot 1972).

Runs were terminated at approximately 10:00 on the following day, at which time counts were made of the number of smolts remaining in the main tank and the number of smolts in each of the eight bucket traps. In all, 40 runs were made between April 18 and May 30, 1988, the entire period of smolt outmigration from Great Central Lake.

Parasitological Studies

The present study involved the 864 smolts that left the tanks in the course of five of the runs conducted between May 5 and May 9, inclusive (Table 1). This period included a sharp rise and the May 8–10 peak in the outmigration of smolts through Robertson Creek. Overall orientation responses were most pronounced and the numbers of fish leaving the tank were largest during this period. For each run, smolts caught in each of the eight bucket traps were preserved in formalin for subsequent examination. These fish were measured, weighed, sexed, and then dissected and examined under 12× magnification for the presence of parasites. Viscera were inspected via respective "squashes" of the stomach, pyloric caeca, intestine, and swim bladder, and forceps were used to tease apart the body musculature. In addition, the eyes of 100 of the smolts were macerated and examined for the presence of parasites. The parasites discussed below account for the known parasite fauna of Great Central Lake sockeye smolts.

The parasites found included the nematode *Philonema oncorhynchi* Kuitunen–Ekbaum, 1933, the cestode *Eubothrium salvelini* Schrank, 1790, muscle and visceral plerocercoids of the cestode *Diphyllobothrium dendriticum* Nitzsch, 1824, and juveniles of an unidentified species of the cestode genus *Proteocephalus*.

Eubothrium salvelini, a pseudophyllidean cestode, infects juvenile sockeye during the first spring or summer of their lake residence when they ingest infected planktonic copepods (Boyce 1974). Infected fish shed *E. salvelini* eggs in their feces the following spring, prior to leaving the lake for their seaward migration. The consumption of eggs by copepods completes the cycle (Boyce 1974). In the present study, the worms were found in the sockeye's pyloric caeca and ranged from 1–40 mm in length.

Diphyllobothrium dendriticum, also a pseudophyllidean cestode, infects a range of fish species, which act as second intermediate hosts (Vik 1964; Andersen et al. 1987; Ching 1988). A number of homoiothermic fish predators, including gulls, bears, wolves, and rodents, act as definitive hosts (Vik 1964; Andersen et al. 1987); eggs shed in the feces of these hosts hatch into free-swimming coracidia, which are infective for copepods, the first intermediate host (Vik 1964). Sockeye become infected with *D. dendriticum* by feeding on infected copepods during approximately a 7 mo period preceding their seaward migration (Vik 1964; Ching 1988). In the salmon the plerocercoid stage was found encapsulated on the viscera — particularly the stomach. They were also found in both encapsulated and unencapsulated forms in the musculature, a finding similar to that of Ching (1988). The capsules were approximately 1 mm in diameter, and unencapsulated individuals ranged from 1 mm to 16 mm in length. Visceral and muscular forms were recorded and analyzed separately, and *D. dendriticum* therefore accounted for two parasite types: "visceral *Diphyllobothrium*" and "muscle *Diphyllobothrium*."

Immature individuals of an unidentified species of *Proteocephalus*, ranging in length from 0.5 to 4 mm, were found in the intestine. The work of Freeman (1964) and Priemer (1987) on *Proteocephalus* infections of trout in Canada and Germany, respectively, suggests that the *Proteocephalus* life cycle is similar to that of *E. salvelini*.

Third stage larvae of the dracunculoid nematode *Philonema oncorhynchi*, ranging in size from 1 to 1.5 mm, were found in the swim bladder wall. Sockeye acquire *P. oncorhynchi* infections during the 5 mo preceding their seaward migration by feeding upon infected copepods of the genus *Cyclops* (Platzer and Adams 1967). The worms remain with the fish for life, eventually migrating from the swim bladder to the coelom in the course of development. Gravid worms, shed by sockeye in the process of spawning, burst upon encounter with fresh water

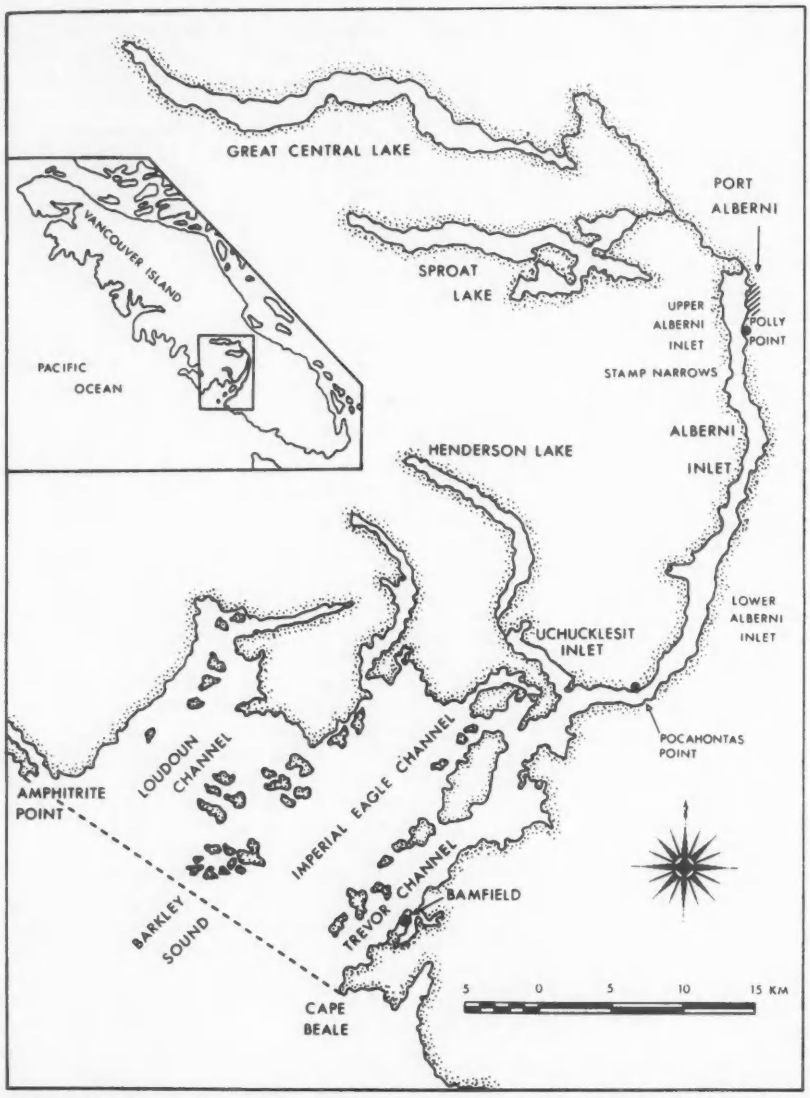

FIG. 1. Map of Great Central Lake area with insert of Vancouver Island/coastal British Columbia.

TABLE 1. Numbers of sockeye smolts involved in each of the five orientation runs.

Date	Tank	Number of fish entering buckets	Number of fish remaining in tank	Total number of fish	Proportion of fish entering buckets
5 May	1	135	258	393	0.34
5 May	2	167	272	439	0.38
7 May	1	102	421	523	0.20
9 May	1	220	257	477	0.46
9 May	2	240	139	379	0.63

and liberate larvae, which are readily available to feeding copepods (Platzer and Adams 1967).

The number of individuals of each of these five parasite types was recorded for each fish. An additional parasite, the myxosporean *Myxobolus arcticus* Pugachev and Khuklov, 1979 (formerly identified as *M. neurobius* Schuberg and Schröder, 1905) occurs in the brain tissue of fewer than one percent of Great Central Lake sockeye smolts (Quinn et al. 1987) and was therefore not considered in the present study. Voucher specimens of helminths are deposited in the National Museum of Natural Sciences, Invertebrate Collection (Parasites), Ottawa, Ontario, Canada with the following numbers: *P. oncorhynchi*: NMCP1989-0431, *E. salvelini*: NMCP1989-0427, visceral *D. dendriticum*: NMCP1989-0428, muscle *D. dendriticum*: NMCP1989-0429, *Proteocephalus*.sp.: NMCP1989-0430.

Statistical Analysis and Experimental Design

A vectorial approach was used to analyze fish orientation. Each fish that left the tank was represented by a vector, of length = 1, whose direction was that of the bucket trap in which the fish was found. The N fish in each run therefore determined N unit vectors, which were in turn summed to yield a resultant vector $\bar{m}^{>}$. The direction of $\bar{m}^{>}$ constituted the "overall" orientation of the fish in the run. The magnitude of $\bar{m}^{>}$, when divided by N, equals "r", which is a measure of the dispersion of fish orientations about the mean direction. The value of r lies on the closed interval [0,1]: if all of the fish in a run left the tank in the same direction (i.e. ended up in the same bucket), then $r = 1$, and, if the fish dispersed evenly with respect to bucket/direction, then $r = 0$. Thus, r approaches unity as fish orientation responses converge towards a single direction. The Rayleigh statistic, $Z = Nr^2$, adjusts r with respect to sample size (N) and is a measure of the extent to which a given smolt distribution among the buckets departs from randomness (Batschelet 1981).

Orientations for some runs were "bimodal" (see e.g. Tables 6 and 8), in that overall orientation aligned along an undirected axis, i.e. two directions 180° apart. In the present case, this appears to have been due to the fact that smolts swimming into the side of a tank and failing to encounter an outlet often swam off in the opposite direction. Bimodal responses are not uncommon in animal orientation studies (Batschelet 1981; Quinn and Groot 1989). In field observations of migrating smolts in Babine Lake, British Columbia, Groot (1965, p. 101) found migration in the direction opposite to the main portion of schools to be "a common phenomenon," and Quinn and Brannon (1982) also found bimodal orientation in experimental studies of Babine Lake smolts. Thus, analysis of each run included computation of $\bar{m}^{>}$, r, and Z under the assumption of both unimodal and bimodal distributions (see Batschelet (1981) for description of techniques), and the tables contain the sets of $\bar{m}^{>}$, r, and Z values giving the higher of the two resulting r (and thus Z) values in each case.

The fish in each run were categorized according to infection status ("+" vs. "−") (see discussion below). Comparison of overall distributions among the outlet buckets of "+" vs. "−" groups was made via a likelihood ratio chi-square test (SAS Institute Inc. 1985), which is the most appropriate type of test for discrete orientation data (Batschelet 1981). These pairwise comparisons were made for each of seven infection categories for each of the five runs, and the preceding vectorial technique was used to analyze the extents to which the distributions of "+" and "−" groups deviated from randomness.

For each run, therefore, the responses of the seven "+" and "−" groups were compared on the basis of three statistics: (1) overall directional orientation, as represented by the direction (but not the magnitude) of $\bar{m}^{>}$, (2) dispersion about the mean direction, as quantified by the magnitude of $\bar{m}^{>}$, and (3) distribution among the eight buckets.

In addition to orientation analyses, two-way analysis of variance (SAS Institute Inc. 1985) was used to compare, for each of the five parasite types: (1) lengths of uninfected and infected fish and (2) weights of uninfected and infected fish.

Results

General Orientation Studies

The proportion of fish exiting a tank in the course of a run reflects the degree of migratory "restlessness" ("zugunruhe" (Dingle 1980)) of smolts (C. Groot, pers. comm.). Over the course of the April 18–May 30 period during which the orientation experiments were conducted, this proportion increased to a peak of 60% just prior to the May 8–10 peak in the numbers of smolts running through Robertson Creek each night, and the proportion subsequently declined to 15–20% toward the end of the migration period.

Orientation of Parasitized Fish

Overall descriptions of the five runs are presented in Table 1, and the responses of smolts in those runs, by bucket, are presented in Table 2. Parasite prevalence and intensity information are presented in Tables 3, 4, and 5. The parasitological analysis permitted the assignment of smolts on the basis of infection intensity (0 worms, 1 worm, etc.) for each parasite, but few fish harboured more than one or two worms of any parasite other than *P. oncorhynchi*. Therefore, for the purposes of statistical analysis, sample size requirements dictated the assignment of fish infection status on the basis of presence ("+") vs. absence ("−") of each of *E. salvelini* ("EU"), visceral *D. dendriticum* ("VD"), muscle *D. dendriticum* ("MD"), and *Proteocephalus* sp. ("PR"). For *P. oncorhynchi* ("PH"), which had a 98% prevalence, the host population was divided as closely as possible into halves: smolts with 0–12 worms ("−"), which accounted for 49.2% of the hosts, and smolts with 13–89 worms ("+"), which accounted for the other 50.8% of the hosts. Table 4 describes the composition of the experimental population as a whole with respect to the overlap of these parasite infection categories. In addition, two "combination" categories included: (1) infection with any cestode (EU^+ and/or VD^+ and/or MD^+ and/or PR^+)

TABLE 2. Final bucket distributions of sockeye smolts for each of the five runs.

Date	Tank	Bucket 1 (0°)	Bucket 2 (45°)	Bucket 3 (90°)	Bucket 4 (135°)	Bucket 5 (180°)	Bucket 6 (225°)	Bucket 7 (270°)	Bucket 8 (315°)
5 May	1	16	11	22	8	12	15	26	25
5 May	2	22	18	10	20	24	20	21	32
7 May	1	6	18	14	11	12	11	11	19
9 May	1	*	17	30	26	31	42	46	28
9 May	2	19	46	26	42	41	20	24	22

*For the 9 May Tank 1 run, the outlet to bucket 1 was inadvertently obstructed; consequently, no fish entered this bucket. Therefore, for the analysis of this run, the experimental tank was considered as having seven outlets ranging in direction from 45° to 315°.

TABLE 3. Prevalence and intensity data for each parasite category for the combined population of smolts from all of the orientation experiments.

Parasite	Prevalence	Intensity range (Number worms)	Mean intensity	SD
E. salvelini	162/863 (19%)	1–14	2.07	± 1.65
D. dendriticum (muscle)	223/864 (26%)	1–6	1.21	± 0.57
D. dendriticum (visceral)	234/863 (27%)	1–7	1.54	± 1.02
D. dendriticum (overall)	382/863 (44%)	1–10	1.65	± 1.17
Proteocephalus sp.	285/863 (33%)	1–13	1.97	± 1.43
Cestodes[a]	606/864 (70%)	1–17	2.52	± 1.95
P. oncorhynchi	848/862 (98%)	1–89	15.42	±11.30
Any parasite[b]	740/862 (86%)	1–92	18.59	±11.66

[a]Includes fish positive for any one or more of the cestodes *E. salvelini*, *D. dendriticum*, and *Proteocephalus* sp..

[b]Includes fish positive for any one or more of the cestodes *E. salvelini*, *D. dendriticum*, and *Proteocephalus* sp. and/or harbouring heavy infection (≥ 13 individuals) of *P. oncorhynchi*.

(="CES$^+$") and (2) infection with any cestode and/or heavy (13–89 worms) infection with *P. oncorhynchi* (="ALL$^+$").

The overall directional response of "−" groups did not differ greatly from those of "+" groups (Table 6). Great Central Lake runs along a 106°/286° axis (Fig. 1), and the overall orientation angle of 221° for the five runs discussed here differs by 65° from that axis. The 9 May Tank 2 run, which was made at the peak of migration and which had the highest proportion of fish exiting the tank (Table 1), was much closer in orientation to the lake axis than were the other runs.

No relative orientation of "−" to "+" groups appeared to be favoured: the sign of "$\theta^- - \theta^+$" was positive in either two or three out of five runs for every parasite category. The cumulative results for the five individual parasite categories were $\theta^- - \theta^+ > 0$ in 11 cases and $\theta^- - \theta^+ < 0$ in 14 cases, and Wilcoxon's signed rank test showed no significant difference ($P>0.05$) between θ^-'s and θ^+'s for this cumulative case (see Table 6). Neither did "−" and "+" groups differ with respect to bimodal responses: seven of 25 "−" groups and nine of 25 "+" groups had bimodal responses, and this difference in responses was not significant ($P>0.05$, chi-square test). The CES$^-$ group had four bimodal responses whereas the CES$^+$ group had 0, and the ALL$^-$ and ALL$^+$ groups each had two bimodal responses (Table 6). Although the orientations of individual "−" groups were closer than their "+" counterparts to the Great Central Lake axis bearing in 17 of the cases and were further away in eight cases, this differential response was not significant ($P>0.05$, chi-square test) (Table 6).

The overall bucket distributions of "+" and "−" groups, as analysed via chi-square, also displayed few differences (Table 7). These two groups differed significantly in their distributions ($P<0.05$) in only three of the 12 run/parasite combinations in which the "+" group sizes were large enough to permit such analysis. The three cases in which significant differences occurred also had the largest differences between θ^- and θ^+, the orientation directions of uninfected and infected individuals (Table 6). Two of these three cases involved *P. oncorhynchi*, and two of the three occurred in the 9 May Tank 2 run with the highest zugunruhe.

Although "+" and "−" groups did not appear to differ significantly with respect to either directional responses or overall distributions, the dispersions of "+" group fish tended toward randomness about their mean orientation vectors, whereas those of "−" group fish tended to depart significantly from randomness (Table 8). For the five individual parasite categories considered for each of the five runs investigated, the Z values for "−" fish groups exceeded their corresponding "+" group values in 20 of 25 cases (see Table 8). The statistical measure of departure from randomness, Z, indicated significant ($P<0.05$) departure in 19 of these 25 cases for "−" fish and

TABLE 4. Frequency of occurrence of various parasite combinations in the sockeye smolts from all runs combined.

Infection status[a] (PH = "low")						Infection status[a] (PH = "high")					
PH[b]	EU[c]	MD[c]	VD[c]	PR[c]	Number of hosts	PH[b]	EU[c]	MD[c]	VD[c]	PR[c]	Number of hosts
L	—	—	—	—	122	H	—	—	—	—	135
L	—	—	—	+	62	H	—	—	—	+	75
L	—	—	+	—	42	H	—	—	+	—	42
L	—	—	+	+	26	H	—	—	+	+	19
L	—	+	—	—	39	H	—	+	—	—	44
L	—	+	—	+	18	H	—	+	—	+	22
L	—	+	+	—	20	H	—	+	+	—	20
L	—	+	+	+	8	H	—	+	+	+	7
L	+	—	—	—	33	H	+	—	—	—	30
L	+	—	—	+	11	H	+	—	—	+	13
L	+	—	+	—	14	H	+	—	+	—	8
L	+	—	+	+	5	H	+	—	+	+	3
L	+	+	—	—	8	H	+	+	—	—	6
L	+	+	—	+	6	H	+	+	—	+	5
L	+	+	+	—	8	H	+	+	+	—	7
L	+	+	+	+	3	H	+	+	+	+	2

[a]See text for key to abbreviations.

[b]"L" = light infection (≤12 worms) and "H" = heavy infections (≥13 worms) (see text).

[c]"−" = uninfected (0 worms), and "+" = infected (1 or more worms).

TABLE 5. Prevalence data (%) for each parasite category for each of the experimental runs.

Parasite	Run				
	5 May Tank 1	5 May Tank 2	7 May Tank 1	9 May Tank 1	9 May Tank 2
E. salvelini	18	10	18	21	23
D. dendriticum (muscle)	24	25	32	27	24
D. dendriticum (visceral)	25	31	26	31	23
D. dendriticum (all)	39	45	48	49	41
Proteocephalus sp.	25	28	25	53	27
Cestodes[a]	61	63	71	84	68
P. oncorhynchi	96	98	99	99	98
Any parasite[b]	87	87	83	91	81
Number of fish in run	135	167	102	220	240

[a]Includes fish positive for any one or more of the cestodes *E. salvelini*, *D. dendriticum*, and *Proteocephalus* sp.

[b]Includes fish positive for any one or more of the cestodes *E. salvelini*, *D. dendriticum*, and *Proteocephalus* sp. and/or harbouring heavy infection (≥13 individuals) of *P. oncorhynchi*.

in only nine of the 25 cases for "+" fish. In both the CES category and the ALL category, Z values for "−" fish exceeded those of "+" fish in four of five cases.

Because the data were constrained to discrete directional categories (e.g. 45° bucket groupings), direct pairwise comparisons of "−" and "+" group Z values for these 25 cases could not be made (Batschelet 1981). However, application of Wilcoxon's signed rank test to the Z values of the 25 "−" groups vs. the 25 values of "+" groups showed the "−" group Z's to be significantly greater ($P<0.002$). For all of the "−" groups and most of the "+" ones, the highest Z values occurred for the runs done during the 9 May migration peak.[2] Moreover, the magnitude of the disparity between "−" and "+" Z values appears to increase fairly consistently with increases in the proportion of fish leaving the tank ("zugunruhe") (Table 8).

Size of Parasitized Fish

Parasitic infection can influence host size (Dombroski 1955; Smith and Margolis 1970; Smith 1973; Henricson 1980), and host size could in turn influence orientation behaviour as a result of its effect on swimming abilities. Two-way analysis of variance in fact revealed that EU+ and VD+ fish were significantly smaller ($P<0.001$) and lighter ($P<0.001$) than EU− and VD−, their respective uninfected counterparts. These findings are in accord with those of Dombroski (1955), Smith and Margolis (1970), and Smith (1973) for *E. salvelini* and reports of various adverse effects of *D. dendriticum* on salmonids (Henricson 1980). None of the other parasite types exhibited significant differences in either of these respects ($P>0.05$). A posteriori tests on the subsample of 701 *Eubothrium*-free fish still showed visceral *D. dendriticum*-infected fish to be significantly smaller and lighter ($P<0.01$) and showed *Proteocephalus*-infected fish to be possibly significantly lighter ($P<0.05$) than uninfected counterparts.

Discussion

Infection status does not appear to play a significant role in overall orientation direction. The "−" and "+" groups manifested broad, overlapping ranges in overall orientation directions (Table 6), as was reflected by the small number of significantly different bucket distributions (Table 7). Bucket distribution patterns (Table 7), which integrate aspects of mean direction and variance about that mean, reveal significant differences, but in only three cases. The salient, unequivocal difference between "+" and "−" status fish was the variation in their orientation: relatively few of the "+" group distributions differed significantly from random, whereas those of "−" groups did so with few exceptions (Table 8), and Z values for "−" groups were significantly larger than those of "+" groups.

The consistent differences between Z^-'s and Z^+'s (Table 8) for individual parasite types were surprising, in that the co-occurrence of other parasite types in fish scored as "−" for a given parasite category would tend to obscure any effects. Just under 40% of cestode infections involved more than one cestode type and less than 10% involved more than two. When heavy *P. oncorhynchi* infections were considered as well, the proportion of multiple infections rose to 58% (Table 4). Statistical analysis of co-occurrences of parasite types indicated that, among all possible parasite type combinations, significant association existed only between visceral and muscle *D. dendriticum*.

This consideration was examined in further detail by computing, for each run and each parasite category, the proportion of "−" individuals that were "+" for any other parasite category. In each case, regression upon these proportions of the quantity "$Z^- - Z^+$" revealed no significant correlations. Moreover, if category overlaps were significantly obscuring any effects, then the aggregations of hosts into the "cestode" and "any parasite" infection categories should have heightened the Z^-/Z^+ contrasts in Table 8. However, this did not occur. Therefore, category overlaps do not seem to be extensive enough to play a significant obscuring role, and the consistent Z^-/Z^+ contrasts in Table 8 would indeed appear to reflect the effects of individual parasite types upon dispersion about mean orientation direction — a finding that holds a fortiori in light of the overlap of the parasite categories.

Either of two different mechanisms, both having negative consequences, could account for the greater variation in orientation direction of "+" group fish. On the one hand, "+" and "−" smolts might simply differ in grouping behaviour. Migrating sockeye smolts school (Groot 1965; Hartman et al. 1967), and, if lightly or non-parasitized smolts were to school more tightly and/or spend more time in schools than more heavily infected smolts, this could readily lead to a more aggregated distribution of the former in the outlet buckets. Infection with *E. salvelini* has been shown to reduce the swimming abilities of smolts directly (Smith and Margolis 1970), and the smaller size of fish infected with *E. salvelini* and *D. dendriticum* would, in and of itself, disadvantage these fish with respect to swimming speed. Moreover, *D. dendriticum* occurs in the host's musculature, and Holmes and Bethel (1972: 131) note that such infections "have been considered debilitating to their hosts."

[2]There were effectively only seven unevenly distributed buckets for the 9 May Tank 1 run (see note, Table 2). Thus, the variance for this run would tend to be lower.

TABLE 6. Overall orientation directions of "+" and "−" groups for each infection category for each of five runs. For each parasite category, "θ^-" and "θ^+" are respective directions of resultant vectors (in degrees) for "−" and "+" infection status groups. Entries separated by a "/" are orientation directions for bimodal (180°) distributions (see text). "θ^-" − "θ^+" is the signed, minimum angular distance between directions θ^- and θ^+: positive and negative values correspond, respectively, to counterclockwise and clockwise movement from the θ^- vector to the θ^+ vector. Also given, in degrees, for each parasite type or grouping are: (1) means and standard deviations of the magnitude of $\theta^- - \theta^+$ for the five runs ("mean $\Delta\theta$") and (2) orientation computed by treating the fish for all five runs as a single run ("All runs").

		Run						
Parasite		5 May Tank 1	5 May Tank 2	7 May Tank 1	9 May[c] Tank 1	9 May Tank 2	All runs	Mean[f] $\Delta\theta$
E. salvelini[a]	θ^-:	105/285*	270*	75/255	219*	136*	214*	
	θ^+:	72/252	0	144/324	208*	31*	346	
	$\theta^- - \theta^+$:	33	−90	−69	11	105		61.6 ± 39.1
Visceral	θ^-:	96/276*	160/340*	88/268	228*	114*	99/279	
D. dendriticum[a]	θ^+:	320	328	169/349	201*	146	234	
	$\theta^- - \theta^+$:	−44	12	−81	27	−32		39.2 ± 26.0
Muscle	θ^-:	295*	276	115/295	214*	124*	207*	
D. dendriticum[a]	θ^+:	90/270	309	11	77/257*	176/356	76/256	
	$\theta^- - \theta^+$:	25	−33	104	−43	−52		51.4 ± 31.1
Proteocephalus	θ^-:	97/277*	287*	39	201*	116*	107/287	
sp.[a]	θ^+:	291	118/298	229	228*	113/293	234*	
	$\theta^- - \theta^+$:	−14	−11	170	−27	3		45.0 ± 70.4
Cestodes[b]	θ^-:	105/285*	168/348	93/273*	86/266	120*	111/291	
	θ^+:	303	311	319	219*	114	238*	
	$\theta^- - \theta^+$:	−18	37	−46	47	6		30.8 ± 18.1
P. oncorhynchi[c]	θ^-:	287*	333*	354	216*	82*	119/299	
	θ^+:	92/272*	223*	154	218*	172*	206*	
	$\theta^- - \theta^+$:	15	110	−160	−2	−90		75.4 ± 66.3
Any parasite[d]	θ^-:	125/305*	302	90/270	195	92*	116/296	
	θ^+:	88/268	163/343	40	218*	142	225*	
	$\theta^- - \theta^+$:	37	−41	50	−23	−50		40.2 ± 11.2
All fish in tank		305	284	94/274	217	117	221	

*Denotes a run for which orientation departs significantly from random (see Table 8).

[a]Comparisons are for uninfected vs. infected smolts.

[b]Category "+" includes fish positive for any one or more of the cestodes *E. salvelini*, *D. dendriticum*, and *Proteocephalus* sp. Category "−" is the complement of category "+".

[c]Comparisons are for "light" infections vs. "heavy" infections (see text).

[d]Category "+" includes fish positive for any one or more of the cestodes *E. salvelini*, *D. dendriticum*, and *Proteocephalus* sp. and/or harbouring heavy infection (≥13 individuals) of *P. oncorhynchi*. Category "−" is the complement of category "+".

[e]For this run, the outlet to bucket 1 was inadvertently obstructed, and, consequently, no fish entered this bucket. Therefore, for the analysis of this run, the experimental tank was considered as having seven outlets ranging in direction from 45° to 315°.

[f]This quantity is the mean of the $\theta^- - \theta^+$'s.

Studies on a range of fish species and parasite taxa amply document the abilities of parasites to interfere with their fish hosts' schooling behaviour (Sindermann 1966; Lester 1971; Guthrie and Kroger 1974; Radabaugh 1980). Because sockeye smolts migrate at approximately their "maximum sustained swimming speed" (Groot 1965: 23), any debilitation could make it difficult for these fish to remain in a school, and, as Pitcher (1986) has noted, fish in schools enjoy increased foraging success and reduced susceptibility to predation relative to their solitary counterparts.

In addition, Pitcher (1986) pointed out that schooling may itself play an important role in navigation. Larkin and Walton (1969: 1372), for example, have demonstrated mathematically that, if the fish in a school jointly orient to some "group mean direction," then navigation error should decrease with increase in group/school size, and Quinn and Fresh (1984), studying homing and straying in chinook salmon (*Oncorhynchus tshawytscha*), found that straying decreased as numbers of returning salmon increased.

However, the experimental results may reflect a direct interference of parasites with their smolt hosts' orientation. Clearly, a finely tuned orientation mechanism is essential to *O. nerka*, given its extensive migratory activity, and, as Groot (1965, 1972, 1981) and Johnson and Groot (1963) have shown, smolts display active orientation capabilities in their seaward migration. This interference could either result from something as immediate as the aforementioned impairment of swimming ability or from some higher order toxicological and/or endocrinological/neurological interaction between host and parasite.

Either or both of these mechanisms could be involved. However, fish swimming out of the tanks and into buckets tended to do so individually and not in schools, and the holes themselves were only 32 mm in diameter. Together with the apparent increase in $Z^- - Z^+$ with increasing zugunruhe, this evidence suggests some kind of interaction between parasites and basic migratory behaviour of smolts.[3]

Relative to the other four parasite types, the tapeworms *E. salvelini* and, to a lesser extent, muscle *D. dendriticum*

[3]It would have been of interest in this respect to have compared the parasite prevalences of all fish entering buckets with those that didn't leave the tank. Unfortunately, it was not possible to obtain the latter specimens.

TABLE 7. "*P*" values for chi-square comparisons of smolt distributions among buckets with respect to infection status. Data are arranged by parasite category and run, as well as the value for all five runs combined. "EU" = *E. salvelini*, "VD" = visceral *D. dendriticum*, "MD" = muscle *D. dendriticum*, "PR" = *Proteocephalus* sp., and "PH" = *P. oncorhynchi*.

	Run					
Contrast	5 May Tank 1	5 May Tank 2	7 May Tank 1	9 May[c] Tank 1	9 May Tank 2	All runs
EU^- vs. EU^+	—[d]	—[d]	—[d]	0.869	0.006*	0.34
VD^- vs. VD^+	—[d]	—[d]	—[d]	0.198	0.119	0.99
MD^- vs. MD^+	—[d]	—[d]	—[d]	0.296	0.665	0.22
PR^- vs. PR^+	—[d]	0.087	—[d]	0.832	0.056	0.12
CES^- vs. CES^+[a]	—[d]	0.730	—[d]	—[d]	0.088	0.76
PH^- vs. PH^+	—[d]	0.001*	—[d]	0.794	0.011*	0.04*
ALL^- vs. ALL^+[b]	—[d]	—[d]	—[d]	—[d]	—[d]	0.23

*The two distributions are considered significantly different ($P<0.05$).

[a]Category "+" includes fish positive for any one or more of the cestodes *E. salvelini*, *D. dendriticum*, and *Proteocephalus* sp. Category "−" is the complement of category "+".

[b]Category "+" includes fish positive for any one or more of the cestodes *E. salvelini*, *D. dendriticum*, and *Proteocephalus* sp. and/or harbouring heavy infection (≥ 13 individuals) of *P. oncorhynchi*. Category "−" is the complement of category "+".

[c]For this run, the outlet to bucket 1 was inadvertently obstructed, and, consequently, no fish entered this bucket. Therefore, for the analysis of this run, the experimental tank was considered as having seven outlets ranging in direction from 45° to 315°.

[d]Sample sizes for these run/parasite combinations were too small for chi-square analysis.

TABLE 8. Grouping response analysis of smolt distributions among buckets by parasite and run: "Z^-" and "Z^+" are respective "Z" statistic values for "−" and "+" infection categories. Also given, in degrees, for each parasite type or grouping are: (1) means and standard deviations of the magnitude of "Z" statistic values for the five runs, and (2) "Z" statistic values computed by treating the fish for all five runs as a single run ("All runs").

		Run							
Parasite		5 May Tank 1	5 May Tank 2	7 May Tank 1	9 May[f] Tank 1	9 May Tank 2	All runs	Mean[g] of Z's	Z^-–Z^+ (signed) Mean SD
E. salvelini[a]	Z^-:	3.26*,[h]	3.07*	1.33[h]	13.12*	9.16*	5.83*	5.99 ± 4.96	3.59 ±
	Z^+:	1.05[h]	1.70	2.34[h]	3.56*	3.36*	0.51	2.40 ± 1.07	4.14
Visceral	Z^-:	3.93*,[h]	3.09*,[h]	2.04[h]	8.60*	6.98*	2.70[h]	4.93 ± 2.76	2.01 ±
D. dendeiticum[a]	Z^+:	0.44	2.90	1.13[h]	9.77*	0.36	1.86	2.92 ± 3.96	3.08 ±
Muscle	Z^-:	4.37*	2.41	0.93[h]	14.74*	9.55*	5.78*	6.40 ± 5.69	4.22 ±
D. dentriticum[a]	Z^+:	1.56[h]	0.68	1.17	6.30*,[h]	1.20[h]	1.36[h]	2.18 ± 2.32	3.97 ±
Proteocephalus sp.[a]	Z^-:	5.89*,[h]	4.26*	1.93	6.23*	8.43*	1.37[h]	5.35 ± 2.42	1.89 ±
	Z^+:	2.90	0.29[h]	2.04	11.55*	0.53[h]	8.79*	3.46 ± 4.65	4.94
Cestodes[b]	Z^-:	6.20*,[h]	2.48[h]	4.28*,[h]	1.46[h]	7.72*	1.09[h]	4.43 ± 2.58	0.13 ±
	Z^+:	2.29	1.61	0.39	15.71*	1.48	4.56*	4.29 ± 6.42	8.26
P. oncorhynchi[c]	Z^-:	4.12*	5.88*	2.62	10.82*	7.40*	1.01[h]	6.17 ± 3.16	2.22 ±
	Z^+:	3.34*,[h]	3.34*	1.45	6.25*	5.35*	8.60*	3.95 ± 1.89	1.49
Any parasite[d]	Z^-:	4.52*,[h]	2.86	1.55[h]	0.96	10.48*	1.42[h]	4.07 ± 3.83	−0.31 ±
	Z^+:	2.59[h]	2.74[h]	0.22	15.88*	2.51	6.16*	4.79 ± 6.29	9.04
All fish in tank	Z :	3.81*	2.92	0.52[h]	16.59*	6.61*	3.94*		
Relative zugunruhe[e]		4	3	5	2	1			

*Result for which Z statistic significant ($P<0.05$).

[a]Comparisons are for uninfected vs. infected smolts.

[b]Category "+" includes fish positive for any one or more of the cestodes *E. salvelini*, *D. dendriticum*, and *Proteocephalus* sp. Category "−" is the complement of category "+".

[c]Comparisons are for "light" infections vs. "heavy" infections (see text).

[d]Category "+" includes fish positive for any one or more of the cestodes *E. salvelini*, *D. dendriticum*, and *Proteocephalus* sp. and/or harbouring heavy infection (≥13 individuals) of *P. oncorhynchi*. Category "−" is the complement of category "+".

[e]Denotes order of runs with respect to proportion of fish that left tank to enter a bucket, with "1" being highest and "5" being lowest.

[f]For this run, the outlet to bucket 1 was inadvertently obstructed, and, consequently, no fish entered this bucket. Therefore, for the analysis of this run, the experimental tank was considered as having seven outlets ranging in direction from 45° to 315°.

[g]Mean of the five Z values (as opposed to aggregating fish from all runs).

[h]Result is for bimodal distribution (see text).

appeared to be somewhat more pronounced in their influence upon smolt orientation. In terms of orientation direction, *E. salvelini* was the only parasite for which "+" fish were consistently further from the 106°/286° Great Central Lake axis than their "−" counterparts, and muscle *D. dendriticum* tied for second in this respect (along with visceral *D. dendriticum*) (Table 6). This parasite also accounted for one of only three cases in which overall bucket distributions differed significantly for uninfected vs. infected groups (Table 7), and, finally, *E. salvelini* was second only to muscle *D. dendriticum* in mean Z^- and mean $Z^- - Z^+$ (Table 8). No one of these distinctions is very great, but they collectively indicate a prominence of *E. salvelini* in its adverse effects upon smolts.

Parasite types may well be expected to differ in the strengths of their influence on orientation. The observed Z^-/Z^+ disparities (Table 8) are also likely to be functions of the particular combination of parasite types present and the numbers and/or mass of parasites per individual. Unfortunately, the relatively small numbers of fish harbouring more than one worm of any given parasite type (other than *P. oncorhynchi*) precluded analysis of orientation in relation to intensity, and sample size constraints precluded an analysis of bucket choice with respect to combinations of the various parasite categories.

Nevertheless, aside from this slight prominence of *Eubothrium* and muscle *D. dendriticum*, the results for the various parasites are surprisingly similar. Regardless of parasite category, "−" groups all displayed considerable variance in orientation direction (Table 6), and, as was previously discussed, for no parasite did "−" and "+" groups display any consistent differences in bucket distribution (Table 7). However, Z^- exceeded Z^+ fairly consistently for all of the parasite categories.

This consistency is surprising in light of coevolutionary considerations. Because transmission of *D. dendriticum* occurs via predation upon infected intermediate hosts, these worms would benefit from any pathogenic effects that would increase their hosts' susceptibility to predation (Godin and Sproul 1988). Although fish can retain *E. salvelini* infections for upwards of 6 mo after commencing seaward migration (Bailey et al. 1989), worms in outmigrating smolts suffer no coevolutionary feedback against pathogenic effects because parasite eggs shed after the smolt host leaves the lake cannot complete their cycle. Mature *Proteocephalus* are rarely found in Great Central Lake sockeye smolts (T. McDonald, Pacific Biological Station, Nanaimo, B.C. V9R 5K6, pers. comm.), and none were found in this study, which suggests that sockeye are probably not important hosts of *Proteocephalus*. In contrast, sockeye are the principal definitive hosts of *P. oncorhynchi*, and parasite transmission is effected only upon completion of the host's life cycle.

In summary, negative effects might well be expected of *Diphyllobothrium*, and perhaps *Eubothrium* and *Proteocephalus*, whereas one would expect *Philonema* to be relatively benign. As the results show, however, each of these helminths appears to exert at least some effect on the orientation of sockeye smolts.

The present study adds a potentially important, albeit subtle effect to the many malevolent influences that endemic parasites can exert upon their hosts — even, as with *P. oncorhynchi*, in the case of an apparently closely coevolved host–parasite relationship. Because abundance, growth rates, and mortality rates are highest during the early phases of the salmonid life cycle, movements occurring during these phases have maximum impact at the population level (Brannon 1981), and, therefore, alteration of these movements could have profound population consequences. Moreover, some studies of migration behaviour have revealed considerable intra-specific variation in directional orientation (Baker 1978), and Schreck (1981: 170) concluded that "there is much variability in migratory behaviour observed in smolts." The results of this study suggest that parasites could account for some of this variation.

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Tidal Influence on Spawning Time of Pacific Herring (*Clupea harengus pallasi*)

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Hay, D. E. 1990. Tidal influence on spawning time of Pacific herring (*Clupea harengus pallasi*). Can. J. Fish. Aquat. Sci. 47: 2390–2401.

Dates of over 17 000 records of Pacific herring (*Clupea harengus pallasi*) spawns from 1951 to 1986 in British Columbia were examined by tidal or lunar phases. The frequency of spawns was significantly higher during neap tides following a new moon. The effect was greatest in spawning areas close to the open Pacific and least in the inside waters of the Strait of Georgia. The mechanism of tidal influence on spawning is uncertain but may involve inhibition of spawning during periods of strong spring tides corresponding to periods of full and new moons. An association between spawning time and tidal phases requires that the annual calendar dates of spawning times change between years. A tidal cycle (or a synodic month) is about 29.6 d, and 12 synodic mo equals 355.4 d, whereas a calendar year is about 365.2 d. Therefore, the dates of tidal phases, such as the spring tide, are either about 10 d earlier or 5 d later relative to the previous year. Other possible consequences of tidal influence include the timing of spawning waves and differences in size composition and egg size between waves.

Les dates consignées de plus de 17 000 fraies de hareng du Pacifique (*Clupea harengus pallasi*) ayant eu lieu entre 1951 et 1986 ont été examinées au regard des phases lunaires et de marée. Les fraies étaient plus fréquentes au cours des marées de mortes-eaux suivant la nouvelle lune. Cette relation était plus marquée dans les secteurs de fraie situés à proximité des eaux du Pacifique et moins marquée dans les eaux du détroit de Géorgie, lesquelles sont relativement isolées du Pacifique proprement dit. On connaît mal le mécanisme par lequel les marées influent sur la fraie, mais il pourrait y avoir inhibition de la fraie durant les périodes de marée de vives-eaux de grande amplitude correspondant aux périodes de pleine et de nouvelle lune. Une telle relation entre les dates de fraie et les phases de marée implique une modification des dates de fraie d'année en année. En effet, la durée d'un cycle de marée (ou mois synodique) est de 29,6 jours, soit 355,4 jours pour douze cycles, tandis que l'année du calendrier est d'environ 365,2 jours. Ainsi, les phases de marée, par exemple les grandes marées, ont lieu environ 10 jours avant les marées correspondantes de l'année précédente ou environ 5 jours après. Les marées pourraient aussi, entre autres effets, influer sur les dates des vagues de fraie et sur la composition selon la taille des individus et la taille des oeufs d'une vague de fraie à l'autre.

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The connection between the spawning time and lunar or tidal cycles has been demonstrated in many organisms (for example, see reviews by Korringa 1947; Gibson 1978; Taylor 1984). Close positive association of spawning with lunar or semilunar phases has been demonstrated in a number of fishes including Atlantic silversides (*Menidia*) (Middaugh 1981; Conover and Kynard 1984), the California grunion (*Leuresthes sardina*) (Thomson and Muench 1976), and several species of *Fundulus* (Taylor et al. 1979; Greeley and MacGregor 1983). Relatively little is known about the effects of tides on herring spawning time. Clarke and King (1985) found that spring-spawning Atlantic herring (*Clupea harengus harengus*) in Milford Haven (U.K.) spawned on neap tides. Spratt (1981) examined 4 years of data on herring spawning in Tomales Bay and San Francisco Bay, California. No relationship to tidal variation was observed in the Tomales Bay spawning, but in San Francisco Bay most herring spawned during the periods when the highest tides fell during darkness.

In British Columbia, Pacific herring (*Clupea harengus pallasi*) spawn during the spring months, mainly during March and April. Spawning often occurs in "waves." Within one spawning location, a single wave or episode of spawns may have a duration of several hours to several days, followed by a later spawning episode in several weeks (Hay 1985). This paper examines the relationship between timing of spawns and tidal cycles.

A consequence of tidal influence is a change in spawning times between years. The tidal cycle is controlled mainly by the lunar cycle. The time between consecutive full moons, or the "synodoc month," is 29.57 d. Twelve synodic months consist of about 355.4 d. Therefore, relative to the length of the 365.2-d calendar years, the full moon and the spring tide it produces occur about 10 d earlier or 5 d later each year relative to the previous year. For example if a spring tide occurred on April 10, or the 100th day of a nonleap year, the next closest spring tide in year $n + 1$ would occur about 10 d earlier, on March 31 or the 90th day of the year on a full moon, or 5 d later on April 15 or the 105th day of the year on a new moon. For convenience in the rest of this paper the "day of the year" will be abbreviated to DOY (this is frequently called Julian Days elsewhere).

Other factors, particularly temperature, influence maturation rates and spawning times (Hay 1985, 1986). Like some other species (Blaxter and Hunter 1982), Pacific herring maturation and spawning time appears to be size dependent, with the older, larger herring spawning earliest. In the North Atlantic the mean

egg size varies among herring stocks as a function of the mean spawning time, with earlier spawning herring having larger eggs (Hempel and Blaxter 1967). Therefore the relationship between egg size, female size, and spawning time was also examined in this study.

Methods and Materials

Data on Spawning Dates

Records of 17 301 British Columbia herring spawns, collected between 1951 and 1986 (Hay and Kronlund 1987), were used in the analyses. Each record usually included the date, location, and spawn dimension (length and width) and intensity. These records were collected by fishery officers and fisheries research staff working throughout the British Columbia coast. The surveys were made from small vessels or by foot, on the shore. The systematic collection of spawning records started in 1929 for some areas of the British Columbia coast. By 1951 almost all areas were routinely surveyed.

Since herring spawn surveys started, there have been some small but significant trends in the data related to changes in survey methods (Hay and Kronlund 1987). Mainly the trends involve a tendency for the mean width of spawns to increase with time (years) and the mean intensity to decrease. These trends can be partially corrected by using a spawn index that holds area-specific vales of width and intensity constant over time while recognizing the area-specific sum of spawn length as an accurate measure of spawn deposition (Hay and Kronlund 1987).

In this paper, which attempts to show a relationship between the timing of spawning and tidal or lunar phases, both the frequency of spawning (i.e. the number of different records of spawning) and spawn index (the cumulative magnitude of spawn deposition expressed in square kilometres of spawn at a uniform intensity) are presented and compared among tidal phases. However, tests of significance are limited to the *frequency* of spawn records. Confining most of the analyses to the date of each spawn record eliminates problems related to time trends in spawn dimensions (Hay and Kronlund 1987).

Comments on the completeness of spawn records are described in Hay and Kronlund (1987). Detailed comments for each of over 100 "sections" (Fig. 1) of the British Columbia coast are presented in Hay et al. (1989a–f). The "section" is the smallest geographic unit used in these analyses. One or more sections can be combined into one of the approximately 30 "statistical areas," which are the next largest geographical scale (Fig. 1). The statistical areas (abbreviated later as SA's) can be grouped into six natural and larger geographical scales called "divisions": (i) the Queen Charlotte Island (SA's 0–2), (ii) the northern coast (SA's 3–5), (iii) the central coast (SA's 6–11), (iv) Johnstone Strait (SA's 12–13), (v) the west coast of Vancouver Island (SA's 21–27), and (vi) the Strait of Georgia (SA's 14–19, 28–29). The largest geographical division is the entire British Columbia coast.

The date of each record was converted to the DOY (adjusted for leap years). For some analyses the lunar cycles were synchronized among years: for each year the DOY was adjusted by adding or subtracting a constant integer to the DOY so that the full moon always occurred on day 100. The adjustment always was made to minimize the absolute change from the original date. Hereafter, these adjusted dates are called "lunar days." Annual changes in the dates of lunar phases are shown from 1971 (when a full moon occurred naturally on DOY 100) to 1980 in Fig. 2. For some analyses, lunar days were cumulated into a 29-d schedule, where all spawns that occurred on the day of a full moon (or spring tide) were set to day 1, spawns occurring on the next day were set to day 2, and so on.

All analyses were made using the Minitab statistical computing system (Ryan et al. 1982). Several methods were used to evaluate the significance of temporal distribution in spawn deposition and the relationship to lunar (or tidal) cycles. A "runs" test was calculated for all data to determine if the timing of spawn deposition was random within the cycle. A significant result indicates that temporal variation in spawn deposition did not occur randomly and that the periodicity was significantly different among the 29 d (Ryan et al. 1982). Runs tests were made on (i) the frequency of independent observations of separate spawns for each day and (ii) on the sum of the spawn index, expressed in units of square kilometres of spawn (Hay and Kronlund 1987). The spawn index reduces time trends in the raw spawn data: specifically a long-term trend for estimates of spawn density to decrease and spawn width to increase. Both these trends are associated with several changes in the spawn survey methodology. The index used here (Hay and Kronlund 1987) is based on area-specific spawn coefficients that are based on average spawn widths and intensities. The frequency of observations of spawns was compared among each day of the 29-d lunar schedule by a chi-square test of homogeneity. A significant difference indicates that the frequency of spawning on some days was significantly greater or less than others.

To determine if periodicity in spawn deposition corresponded to tidal or lunar phases, the number of spawn records in each of the four phases of a synodic month (i.e. two spring tides and two neap tides) was calculated. The duration of a synodic month, 29.5 d, is not divisible into even numbers, so there is some unavoidable error in the classification of certain days that could, with equal validity, be the last day of a neap versus the first day of a spring tide. There also is some small but unavoidable error associated with variation in the duration of lunar phases (between 13 and 15 d) that is caused by the elliptical lunar orbit. The lunar phase is shorter (13 d) when the moon is closest to the earth (perigee) and longer (15 d) when farthest from the earth (apogee).

To account for the lunar (and tidal) irregularity, four symmetrical lunar months were defined with durations of 32, 28, 28 and 32 d, respectively. Lunar day 100 was defined as the day with a full moon. A natural lag of several days delays the time of maximal spring tides until several days after the date of full or new moons (Thomson 1981). Therefore the tidal cycle corresponding to lunar day 100 was set to begin on day 98 and extend 7 d to day 104, followed by a 7-d neap tide from day 105 to 111. In a similar way, four full tidal cycles were defined as indicated in Fig. 3. This idealized regime has a total of 120 d with exactly 30 in each of the four tidal phases: (two spring tides on new and full moons and two neap tides). The four idealized lunar cycles extend from lunar day 38 (approximately early February) to lunar day 157 (late May or early June) and include 16 977 (98.1%) of the total 17 301 observations.

To compare spawning periodicity among areas, the frequency of spawns was plotted as a function of lunar day. A "smoothing" function (Ryan et al. 1982), which uses a combination of running medians of the second, third, fourth, and fifth consecutive values and a running average where

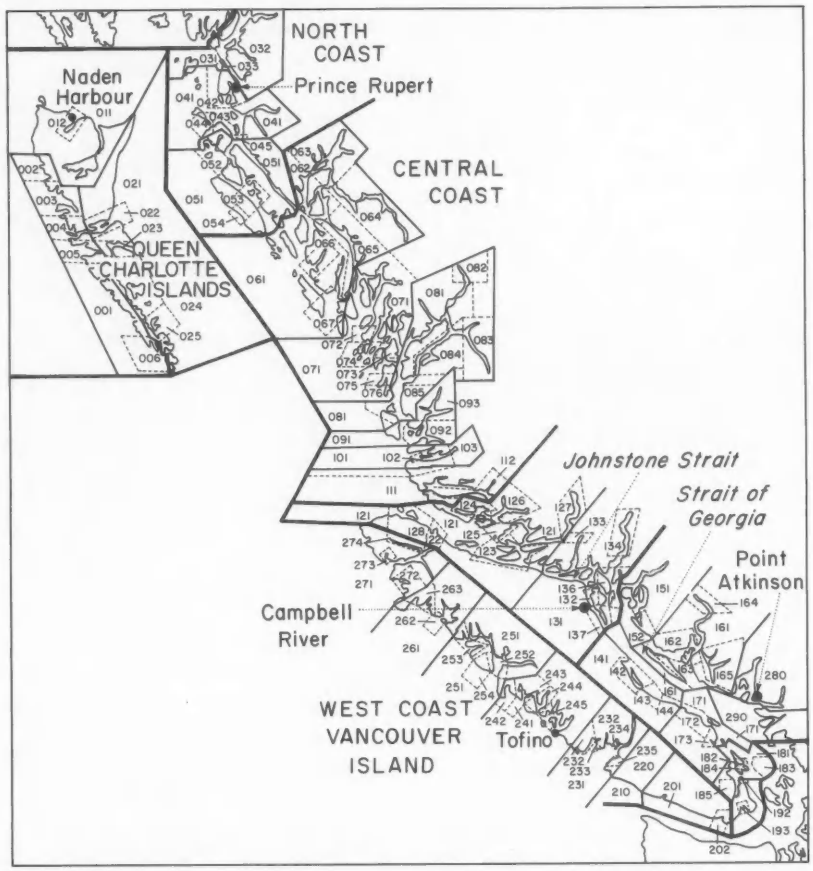

FIG. 1. British Columbia coast. Heavy lines separate six major geographical divisions (Queen Charlotte Islands, north coast, central coast, Johnstone Strait, Strait of Georgia, and the west coast of Vancouver Island). Lighter lines indicate the statistical areas and broken lines separate the smallest areas called sections. Sections are identified by a three-digit number; the first two digits identify the statistical area.

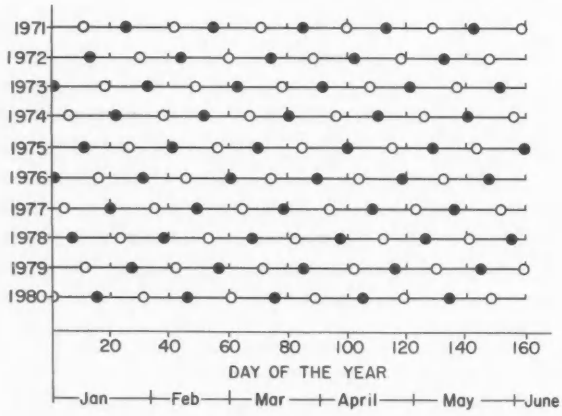

FIG. 2. Approximate dates of full and new moons for 10 yr from 1971 to 1980. In 1971 the full moon fell on DOY 100. The open and solid circles represent full and new moons, respectively.

$z(t) = 0.25\ y(t-1) + 0.5\ y(t) + 0.25\ y\ (t+1)$, was used for illustration in Fig. 4 and 5 but the smoothed data were not used for statistical comparisons.

Field Collections

Samples of spawning herring were collected by small experimental purse seines during successive spawning waves in Barkley Sound in 1982 and Lambert Channel in 1983. Research staff were resident in the area during those years and were able to take samples for several consecutive days within each day of both spawning waves. Each sample consisted of several hundred fish. Fish were measured to the nearest millimetre; total and gonad weights were determined to the nearest gram. The gonosomatic index (GSI) was estimated as the percentage of total body weight made up of gonads.

Female Size – Egg Weight Relationship

Eggs from ripe females collected in Barkley Sound in 1982 were extruded individually on glass slides within hours of the capture of the fish. The eggs were dried in air and further dried in a drying oven for 24 h at 70°C prior to weighing, to the

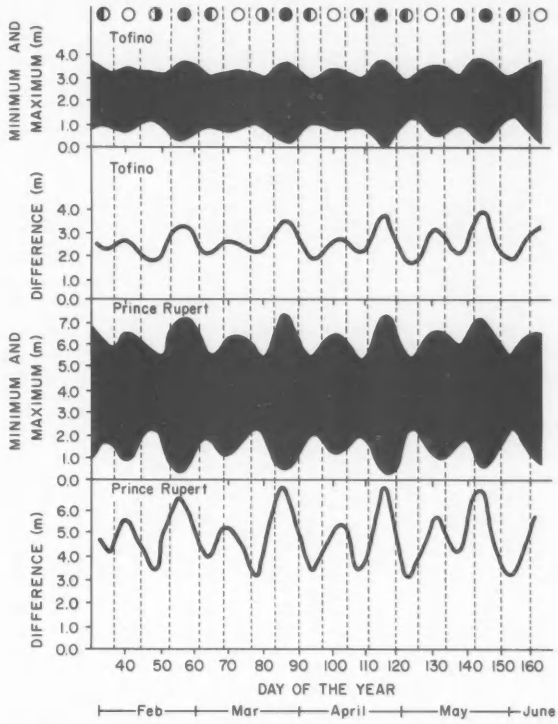

FIG. 3. Daily maximum and minimum tidal heights (solid) and differences between the maximum and minimum for Tofino on the west coast of Vancouver Island and Prince Rupert on the north coast from February to early June 1971. Vertical lines separate periods of spring tides (on the new and full moons) from neap tides. Four complete tidal cycles are shown. Open and solid circles represent periods of spring tides; half-solid circles represent periods of neap tides.

nearest microgram, on a Cahn electrobalance. Ten eggs from each female were weighed individually and the mean was used as the estimate of egg weight for each female.

Results

Relationship between the DOY and the Synchronized Lunar Days

The cumulative spawn index for each DOY, pooled for all years from 1951 to 1986, is shown for a representative statistical area (Area 23) in Fig. 4a. There is no periodicity evident in this distribution. The effects of the conversion of the DOY to synchronized lunar days and of the smoothing function are shown in Fig. 4b and 4c, respectively. Figure 4b, the cumulative spawn index by synchronized lunar day, shows three successive peaks, each corresponding with a period of neap tides (half moons). This trend is shown more clearly when the data are smoothed as in Fig. 4c. The smoothing function was used only for graphical display and not for quantitative analysis. The percent frequency distribution of spawn records is shown in Fig. 4c. Here, only the number of *records* is considered. The percentage of all records occurring on any given day within the 29-d lunar cycle is shown as the broken line. The same data, after smoothing, are indicated by the solid line.

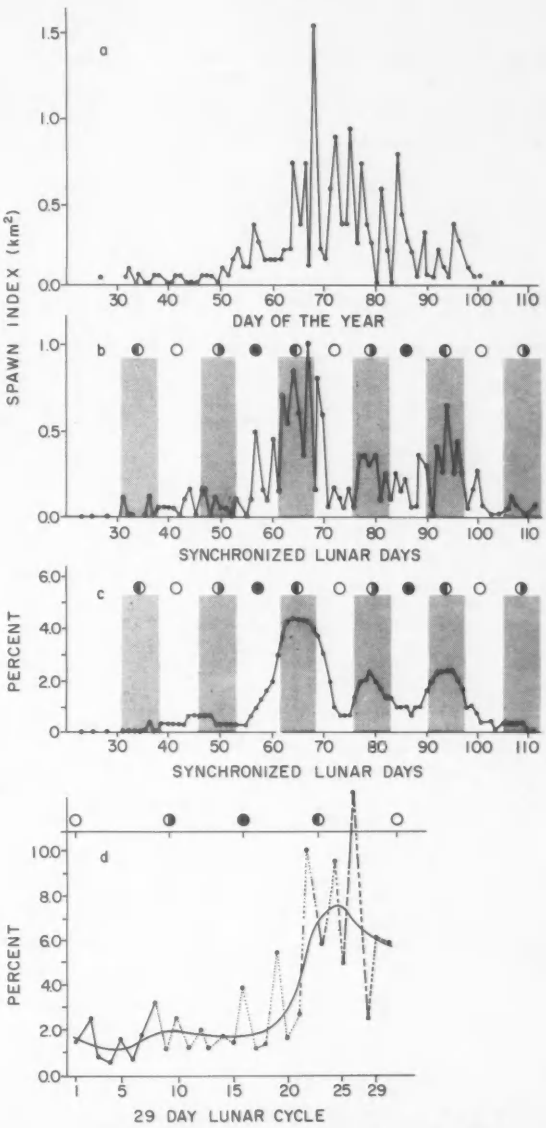

FIG. 4. (a) Spawn index, by DOY, pooled for all years from 1951 to 1986, from statistical area 23 on the west coast of Vancouver Island. (b) Spawn index, by lunar day, for the same area. The tidal cycles of all years were synchronized so that the full moon always fell on day 100. Shaded sections indicate periods of neap tides. (c) Spawn index after smoothing, plotted as a daily percentage of all spawn deposited in area 23. (d) Frequency of spawns per day within a 29-d lunar cycle. Day 1 represents day of the full moon and period of spring tides. Broken lines join points indicating the percentage of all spawns that occurred on each day in the 29-d lunar cycle. The solid line represents the smoothed curve (as described in the text).

Relationship between Lunar Stages, Tidal Cycles, and Spawning Time

When all spawn records were cumulated into a 29-d lunar schedule, there were significant differences in the frequency of

TABLE 1. Number of spawning records and cumulative spawn index for major geographical divisions and statistical areas of the British Columbia coast. A chi-square test of homogeneity was used to compare the number of spawns per day within a 29-d lunar cycle (with day 1 as the full moon). The temporal pattern of variation in the number of spawns per day and the spawn index were compared with a runs test (see text for explanation). QC, NC, CC, JS, GS, and WCVI refer to geographic divisions Queen Charlotte Islands, north coast, central coast, Johnstone Strait, Strait of Georgia, and the west coast of Vancouver Island, respectively.

Area	Number of records	Sum spawn index	Chi-square (29 df)	*P* runs in number	*P* runs in index
Coast	17 301	311.7	786.18	0.002	0.018
Division					
QC	2 405	27.5	356.87	0.006	0.048
NC	1 958	38.7	393.73	0.061	0.285
CC	4 415	38.0	440.91	0.015	0.097
JS	1 464	18.0	153.86	0.370	0.097
GS	3 373	132.3	132.25	0.018	0.142
WCV	3 686	57.2	679.35	0.002	0.607
Statistical area					
0	740	3.9	175.60	0.607	0.015
1	149	2.9	103.28	0.695	0.596
2	1 516	10.4	482.44	0.001	0.048
3	275	4.1	217.37	0.426	0.129
4	927	22.2	355.35	0.002	0.231
5	756	12.4	241.58	0.895	0.347
6	878	10.2	313.91	0.023	0.505
7	2 044	16.0	380.25	0.370	0.231
8	820	5.1	489.53	0.802	0.802
9	289	5.0	164.46	0.481	0.224
10	288	1.5	127.47	0.089	0.004
11	96	0.3	63.55	0.394	0.778
12	862	14.4	117.34	0.201	0.201
13	602	3.6	128.30	0.349	0.089
14	567	60.7	87.83	0.792	0.889
15	247	15.3	96.11	0.706	0.706
16	344	5.7	47.84	0.845	0.162
17	1 048	37.8	131.26	0.002	0.420
18	1 035	5.9	89.33	0.192	0.885
19	10	0.0	—	—	—
28	46	0.0	22.01	0.063	0.398
29	76	6.9	73.02	0.018	0.894
20	7	0.0	—	—	—
22	1	—	—	—	—
23	963	15.2	768.36	0.016	0.061
24	1 467	12.8	445.28	0.041	0.607
25	703	19.8	181.07	0.978	0.061
26	171	2.1	33.69	0.895	0.530
27	374	7.4	166.92	0.527	0.716

spawns compared among days. When examined over large geographical areas such as divisions and statistical areas, the differences in the number of spawns per day usually were significant (Table 1). Significant runs occurred in all of the geographic divisions although they were less pronounced than when examined at the level of the statistical area. This indicates that there were significant differences in the amount of deposited spawn when compared among days within a 29-d tidal cycle.

In general, the frequency of spawns and the spawn index were greatest during the neap tide following a new moon but the results are not consistent among areas (Table 2). The effect of tidal variation on spawn frequency was less pronounced in statistical areas corresponding to the inside waters of Johnstone Strait and the Strait of Georgia than it was on the outside waters of the north and west coasts of Vancouver Island.

Comparison of the timing of spawns, by synchronized lunar days, showed that spawning cycles vary among areas and the modes do not correspond exactly to the spring – neap regime, even though an approximate 2-wk periodicity is evident in most sections. For instance, there is a strong mode during the neap tide prior to the full moon that is consistent from statistical area 3 to area 8 (Fig. 5a). For unknown reasons, the apex of the highest mode shifts to the right (closer to the spring tide) as the latitude decreases. This shift also occurs in the adjacent, smaller modes. In general, these modes correspond to different waves of spawning, although there are rarely more than two waves of spawning within sections (the smallest geographical unit). The Queen Charlotte Islands (Fig. 5b) have a mixed pattern of spawning although statistical area 2 on the southeast, which is the most significant spawning area of the Queen Charlotte Islands, has a periodicity nearly identical with that of statistical area 6, on the adjacent central coast. The tidal periodicity for the two areas is also similar. Within the inside waters of the Strait of Georgia and Johnstone Strait there is no apparent tidally influenced trend in the pattern of spawn deposition except for the east side, areas 15 and 16 (Fig. 5c), which have most spawning on the neap tides. Areas 23 and 24 on the southwest coast of Vancouver Island have strong spawning modes during the neap tides prior to the full moon, but the pattern changes in the northwest coast of the island (Fig. 5d).

Comparison of Fish Sizes among Spawning Waves

Both in 1982 in Barkley Sound (west coast of Vancouver Island) and in 1983 in Lambert Channel (Strait of Georgia) the mean weights and lengths of females and males were less for the second wave of spawners (Table 3). If catches of spawning herring are made during the early spawning waves, they can include immature fish, and catches made on later spawning waves can include some spent fish. This association of immature and spent fish can confound attempts to compare sizes among *spawning* fish from different waves. However, by restricting the size comparisons to fish with a GSI of 20, spent and immature fish are eliminated from the analyses. The trend for larger fish in the early spawning waves held for comparisons of fish with GSI estimates of 20 or more as well as for comparisons made among all fish with lower GSI's. This indicates that the results were not affected by mixtures of spawning, spent, and immature fish.

Egg Weight – Female Size Comparison

Dry egg weight (milligrams) increased significantly with female length (millimetres) (Fig. 6). The regression equation (egg weight = $0.085 + 9.41 \times 10^{-4}$ (length)) accounts for about 32% of the variation in egg weight ($R^2 = 32$). Based on this equation, egg size would increase by about 22% from about 0.254 mg in a small 180-mm female (approximately the length of recruits) to about 0.311 mg in a larger, older female of 240 mm (a moderately larger repeat spawner). This equation, however, applies only to Barkley Sound in 1982 and may not apply in different years or in different areas. However, positive covariance of female size and egg size in Pacific herring has been shown by others (Kingston 1982).

TABLE 2. Frequency of spawning and spawn index (square kilometres) in different tidal cycles of statistical areas, organized by geographic divisions (Fig. 1). The numbers of spawn records are compared among four lunar phases. Two neap tides are distinguished: the neap before spring tides on the new moon (NBNM) and the neap before spring tide on the full moon (NBFM). Within each statistical area the highest frequency and index among the four phases are underlined. Records from 1951 to 1986 were pooled. The null hypothesis that the expected frequencies in each tidal phase are equal was tested by chi-square analyses. Significance at the 0.05 and 0.01 probability levels is indicated by one or two asterisks.

Statistical area	Neap (NBNM)		Spring, new moon		Neap (NBFM)		Spring full moon		Total		
	No.	Index	No.	Index	No.	Index	No.	Index	No.	Index	Chi-square
					Queen Charlotte Islands						
0	131	0.74	181	0.97	236	1.23	192	0.92	740	3.86	30.2**
1	30	0.51	35	0.70	22	0.64	24	0.44	111	2.29	3.8
2	416	5.23	224	2.99	438	7.44	387	4.82	1 465	20.48	77.2**
					Prince Rupert district						
3	50	0.67	83	1.13	76	1.35	65	0.94	274	4.09	9.1*
4	123	4.65	271	5.28	311	6.35	209	5.67	914	21.95	88.1**
5	157	2.78	195	2.99	205	3.84	195	2.77	752	12.38	7.2**
					Central coast						
6	252	2.33	120	1.77	289	3.59	189	2.44	850	10.13	77.7**
7	436	2.96	402	3.53	666	5.44	540	4.08	2 044	16.01	82.9**
8	203	1.13	122	0.98	258	1.29	168	0.83	751	4.23	52.6**
9	66	0.95	49	0.98	72	1.27	102	1.77	289	4.97	20.3**
10	84	0.30	68	0.31	71	0.53	65	0.38	288	1.52	2.9
11	28	0.09	27	0.10	26	0.05	14	0.04	95	0.28	5.4
					Johnstone Strait and Strait of Georgia						
12	177	2.89	194	3.46	286	5.11	205	2.98	862	14.44	32.5**
13	100	0.49	145	0.87	143	0.88	213	1.38	601	3.62	43.5**
14	117	12.27	169	16.80	152	18.81	129	12.79	567	60.67	11.4*
15	79	4.60	67	3.90	54	4.85	46	1.96	246	15.31	10.3*
16	62	1.02	100	1.51	99	1.37	81	1.76	342	5.66	11.7*
17	176	6.81	216	8.08	351	11.49	302	11.38	1 045	37.76	72.8**
18	205	1.37	227	1.33	248	1.42	292	1.51	972	5.63	17.0*
28	6	0.08	10	0.17	13	0.21	17	0.25	46	0.71	5.7
29	27	2.18	22	1.84	12	1.54	12	0.96	73	6.52	9.2*
					West coast Vancouver Island						
23	139	2.73	160	2.77	512	7.29	138	2.16	949	14.95	425.6**
24	300	2.37	287	2.06	556	5.09	318	3.18	1 461	12.70	134.1**
25	159	5.26	140	4.32	215	5.41	181	4.35	695	19.34	17.9**
26	39	0.46	46	0.32	52	0.81	34	0.48	171	2.07	4.4
27	58	1.00	78	1.83	96	1.82	142	2.76	374	7.41	41.3**
Sum	3620	65.87	3 638	70.99	5 459	99.12	4 260	73.00	16 977	308.98	

Discussion

The spawning dates recorded by fishery officers often may be in error by a day or two, and sometimes more. Such errors, however, would occur randomly throughout the data and their effect would be to dampen real modes in spawn timing. The analyses indicate that most spawning occurs during neap tides, particularly those following new moons, but there are exceptions. One notable exception was in Barkley Sound in 1982 and another was in Lambert Channel in 1983 at the time of the field work. At both times the major spawns coincided with spring tides although spawning in Barkley Sound usually occurs on the neap tide. Clearly, the association between lunar phases or tidal cycles and the initiation of Pacific herring spawning is not exact. In 1982, however, spawning began on the full moon spring tide, which usually is smaller than those coinciding with the new moon (Fig. 3). Therefore, rather than choosing to spawn on neap tides, instead herring may avoid spawning during spring tides, especially the spring tide associated with the new moon. Southern British Columbia herring populations tend to spawn mainly on the neap tide before full moons occurring during early March, around day 70 in Fig. 5c. In contrast, northern herring populations spawn about one lunar month later, mainly on the neap tide before the full moon in early April, around day 100 in Fig. 5a.

The effect of tidal influence over spawning varies geographically. The tendency for spawning to begin during periods of neap tides is most pronounced in the outside waters and least in the inside waters, such as Johnstone Strait and the Strait of Georgia. Also, there are substantial differences in tidal height, with the greatest amplitude in northern waters (Fig. 7). Further, there are geographic differences in the frequency and amplitude

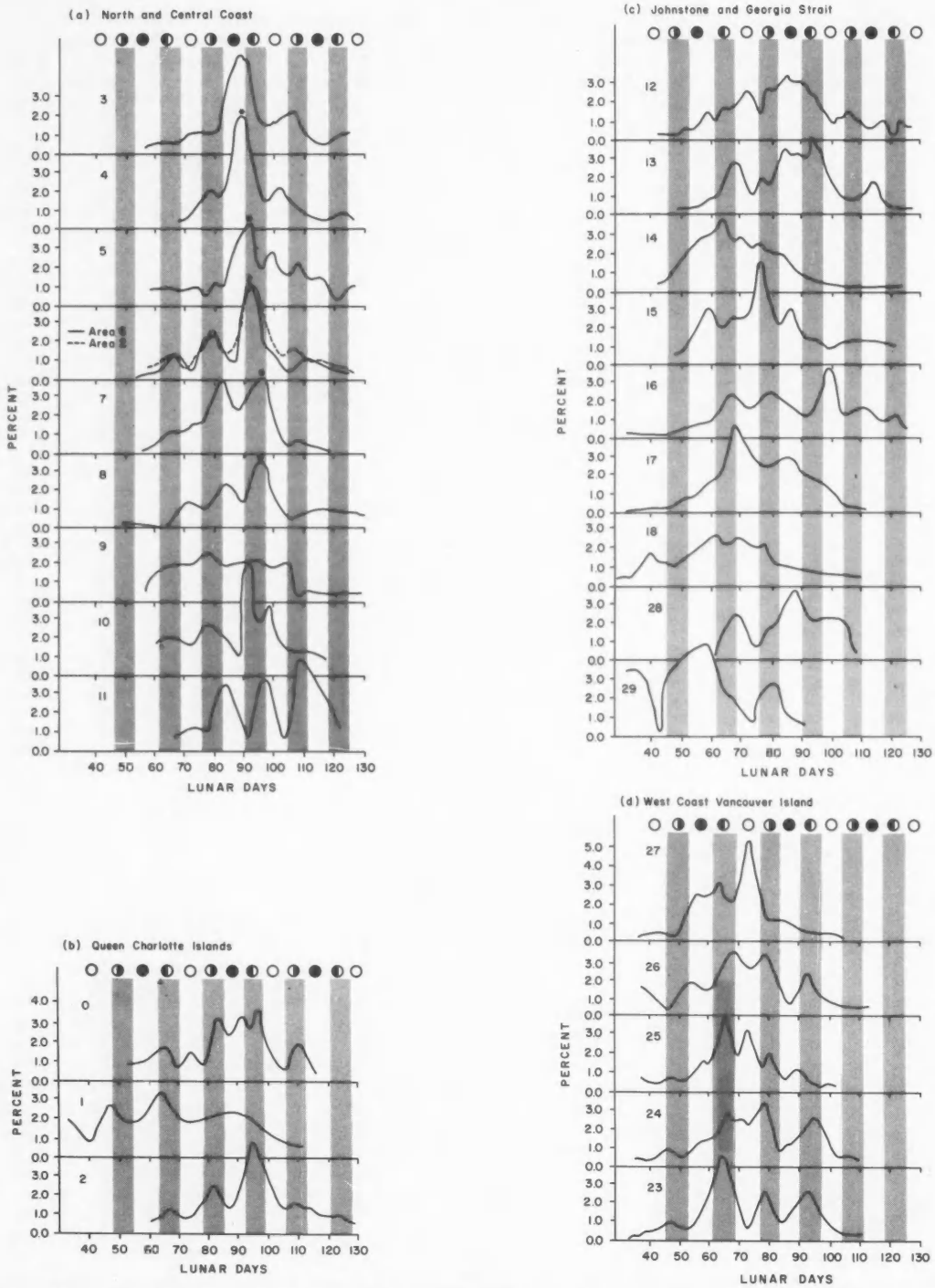

FIG. 5. Smoothed spawn index, plotted by lunar day, shown as a percentage of the total spawn index (all spawn records summed over all days of all years from 1951 to 1986). Periods of neap tides are indicated by shading. Full and new moons are indicated by open and solid circles and half moons by half-solid circles. (a) North and central coasts (statistical areas 3–11; (b) Queen Charlotte Islands (areas 0–2); (c) Johnstone Strait (*Fig. 5 concluded next page*)

TABLE 3. Comparison of mean length (mm), weight (g), and gonosomatic index (GSI) between the first and second spawning waves in Barkley Sound, 1982, and Lambert Channel, 1983. The first and second waves occurred about March 6–10 and March 24–25, 1982, and February 27 – March 3 and March 13, 1983, respectively. Standard deviations are shown in parentheses. Single and double asterisks indicate significant differences at the 0.05 and 0.01 probability levels, respectively, of mean estimates for each sex, between waves (ns = not significant).

	All GSI's				GSI > 15				GSI > 20			
	n	Length	Weight	GSI	*n*	Length	Weight	GSI	*n*	Length	Weight	GSI
						Barkley Sound, 1982						
Female, wave 1	450	199.2 (19.2)	118.9 (32.2)	22.6 (6.4)	400	200.5 (19.4)	122.5 (31.9)	24.4 (4.3)	318	203.5 (18.1)	129.3 (32.0)	26.1 (5.3)
Female, wave 2	900	188.8 (26.7) **	94.3 (32.1) **	17.8 (8.4) **	602	190.4 (28.2) **	106.1 (33.2) **	23.2 (5.0) **	440	193.7 (29.8) **	107.5 (33.4) **	25.3 (4.3) **
Male, wave 1	460	195.0 (21.0)	105.5 (28.3)	14.1 (5.7)	239	200.4 (29.6)	118.6 (27.6)	20.1 (3.0)	76	206.6 (20.1)	131.6 (31.8)	22.5 (2.9)
Male, wave 2	1388	182.0 (20.4) **	79.3 (27.9) **	8.9 (5.8) **	252	189.6 (20.4) **	98.0 (30.8) **	16.6 (3.3) **	76	194.0 (21.4) **	106.2 (35.0) **	22.6 (2.9) ns
						Lambert Channel, 1983						
Female, wave 1	1480	207.1 (18.4)	124.9 (35.6)	22.4 (6.2)	1297	208.7 (18.2)	128.6 (34.4)	23.3 (4.5)	994	209.8 (17.3)	131.6 (33.8)	25.7 (3.5)
Female, wave 2	190	199.2 (18.0) **	100.5 (30.1) **	18.5 (5.7) **	159	199.5 (17.7) **	101.2 (30.0) **	20.7 (3.6) **	102	202.5 (16.6) **	105.7 (30.7) **	23.7 (2.1) **
Male, wave	2699	199.4 (21.0)	106.0 (28.3)	14.5 (5.7)	1600	201.0 (29.6)	111.4 (27.6)	20.1 (3.0)	1069	201.0 (20.1)	110.7 (31.8)	22.8 (2.9)
Male, wave 2	540	199.2 (19.5) ns	98.0 (29.6) **	12.7 (6.4) **	301	199.0 (20.2) ns	99.2 (32.5) **	19.7 (3.5) *	216	198.7 (20.7) ns	97.6 (34.2) **	23.2 (2.6) ns

of semidiurnal tides between the inside waters of the Strait of Georgia and tides on the outer coast.

Thompson (1981) pointed out that the 29.6-d synodic tidal cycle is most pronounced along the open waters of the outer British Columbia coast. In contrast, within enclosed waters such as the Strait of Georgia, other types of tidal forces with different periodicities, usually 27.3 d, obscure the 29.6-d periodicity of the synodic tide. The most important influences are the anomalistic tides (related to the moon's elliptical orbit) and declinational tides (related to the tilt of the moon's orbit relative to the axis of the equator). Therefore, possible spawning initiation cues from tidal amplitude or current velocity may be obscured or more complex in inside waters relative to outside, exposed waters. In this regard, it appears that the peak spawning activity within the Strait of Georgia is less clearly associated with the tidal phases than elsewhere on the coast. The role of tidal currents as a factor in the initiation or prevention of spawning is a subject for future investigation.

Tides are known to affect behaviour of herring at other seasons. Tester (1938) showed that catches of Pacific herring in the 1930's (when there were virtually no regulations or effective quotas) were larger during spring tides. Also, Savage and Hodgson (1934) showed that the fall catches of herring off East Anglia, in the North Sea, were greatest during periods of full moons. Blaxter and Holliday (1963) briefly reviewed the possible effects of lunar influence on herring. They listed several possible mechanisms of direct or indirect lunar effects on clupeids: (1) an increase in activity level, particularly in shallow water, caused by the light of the moon; (2) moonlight may be sufficiently bright to cause changes in shoaling patterns (or change net avoidance capacity); (3) the tidal effect associated with lunar phases might have a variable influence on fish excitability, on their horizontal or vertical migration, or on their shoaling behaviour; (4) moon or tidal effects may affect predator–prey relationships.

Of these four explanations, the first two, invoking an effect of increased illumination, probably can be dismissed as factors consistently affecting Pacific herring during spawning because the British Columbia coast usually is cloudy during the herring spawning season. For instance, the Canadian Atmospheric Environment Service (Environment Canada 1980, 1981, 1982) publishes monthly data on observed sunshine as a percentage

FIG. 5. (*Concluded*)
and Strait of Georgia (areas 12–19, 28–29); (d) west coast of Vancouver Island (areas 23–27). In Fig. 5a, the modes of the neap tides, between areas 3 and 8, are distinguished with an asterisk to show the gradual geographic shift in the mode. The plot for area 2, Queen Charlotte Islands, is superimposed on area 6 to show the close resemblance of spawning time patterns between the two areas.

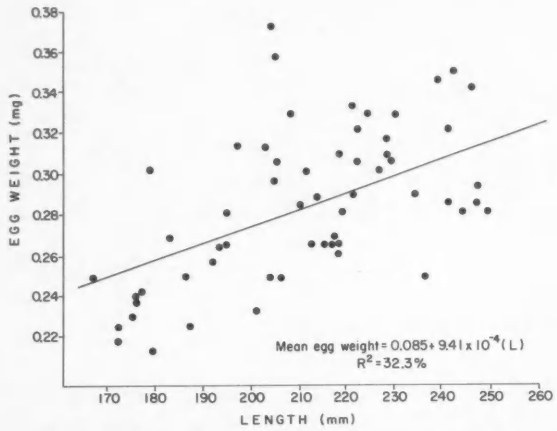

FIG. 6. Female length – egg weight relationship. Dry egg weight is plotted by female length.

of the total possible sunshine for various locations on the British Columbia coast and elsewhere. This estimate would also be representative of the total proportion of time that a location is under lunar illumination. In the years 1980–82, the average estimates for Prince Rupert and Tofino were, respectively, 30 and 38% for the months of February, March, and April. Further, it is not clear how the moon could directly affect predator–prey relationships (the last explanation) except by increased illumination. Again this may be an important factor at some times of the year but it probably is unlikely during the usually cloudy spawning period. The third explanation appears to be consistent with the evidence; that is, herring respond to differences in tidal currents to move into shallow water over the spawning grounds. This was the conclusion of Clarke and King (1985) who studied a population of spring spawning herring in Milford Haven (U.K.) where herring gillnet catches were higher during periods of neap tides. They used echo-sounder data to confirm that the difference in catches was attributable to differences in fish availability — herring did not move into the shallow water during the spring tides as readily as they did during neap tides. They described the prespawning aggregation of Milford Haven herring as moving into the shallow water, close to the bottom, during a neap tide, and spawning on the neap tide. Following spawning the herring moved out quickly and few spent herring were taken on the subsequent neap tide.

Spratt (1981) also described a possible tidal influence on the spawning of Pacific herring in San Francisco Bay although there, herring appear to spawn mainly at night during periods of high tides. These observations, combined with those presented in this paper, suggest that tidal currents may indeed be factors affecting herring movements and choice of spawning area. However, it is not clear that herring specifically select neap tide or avoid spring tide conditions. Rather, local current patterns may be important and it is conceivable that some specific locations which are chosen during neap tides are ignored during spring tides, and vice versa. In some areas, meteorological conditions, particularly winds that affect tidal height and current velocity, may have a relatively strong effect on spawning initiation.

Prespawning herring must use a variety of environmental signals to initiate spawning and perhaps to select spawning grounds. Obviously, temperature is important in some locations

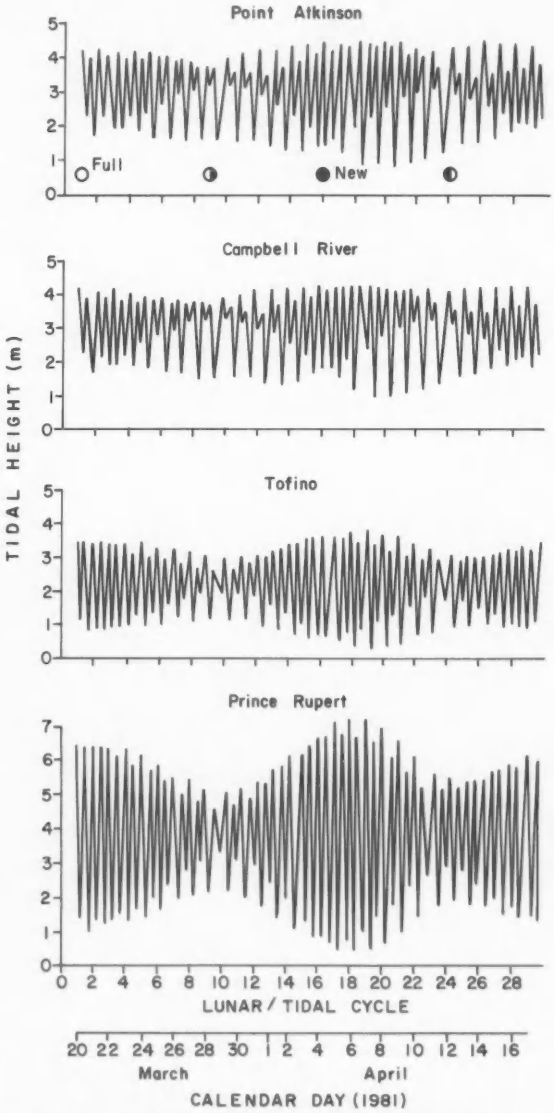

FIG. 7. Tidal amplitude and variation in diurnal tides at different locations on the British Columbia coast. There are complex time lags within the Strait of Georgia (indicated by Point Atkinson and Campbell River) which alter the synchrony of tides in inshore locations. On outer coastal locations (such as Tofino and Prince Rupert) the tides are in synchrony although the amplitude is greater in northern waters.

(Hay 1985; Messieh 1975). Salinity cannot be ruled out but there are virtually no spawning records of British Columbia herring spawning in fresh or estuarine water, even though the eggs are remarkably euryhaline (Alderdice and Velsen 1981). In British Columbia, herring may have evolved to spawn during neap tides as a means of avoiding rapid tidal currents which could interfere with spawning success. Faster currents may reduce the probability of fertilization as a function of dilute sperm. Alternatively, rapid tidal currents could interfere with

the deposition of the adhesive eggs on submerged vegetation, particularly if the vegetation was moving in response to current.

The time between successive neap tides, about 14 d, is similar to the incubation period of herring at ambient temperatures and salinities. Alderdice and Velsen (1971) determined separate equations describing relationships between (1) hatching time or percent viable hatch, temperature, and salinity and (2) percent development per day as a function of temperature. Normally salinities for most of the known herring spawning areas are about 29 ppt and if the Alderdice and Velsen equations are rewritten, holding salinity constant at 29 ppt, they are (1) VH $= 1.348 + 17.760\,T - 0.968\,T^2$ and (2) $\%D = 0.765 + 0.437\,T + 0.024\,T^2$, where VH is percent viable hatch, T is temperature (degrees Celsius), and $\%D$ is percentage of daily development. Development is complete (100%) at hatching time so this equation can be rearranged to express days to hatching: $HT = 100/(0.765 + 0.437\,T + 0.024\,T^2)$ where HT is days to hatching (Fig. 8a). From these equations the relationship between temperature, percent viable hatch, and hatching time is easily determined (Fig. 8b). The incubation time for optimal hatch at ambient temperatures is about 15 d — identical to the time between neap tides. The correspondence between optimal hatching time and tidal periodicity could be spurious, but it also could be an indication that spawning and hatching times are adapted to be compatible with tidal cycles.

A specific advantage to spawning on a neap tide is that hatching and larval release would correspond with lower tidal amplitudes and slower velocities at hatching. Further, because about one third of Pacific herring egg deposition is in the intertidal zone (Haegele et al. 1981), spawning on a neap tide ensures that eggs are confined to the lower elevations within the intertidal zone. Haegele et al. (1981) found that egg mortality was relatively higher at higher intertidal elevations, so eggs deposited in the high intertidal zone, during a spring tide, could be at a disadvantage. It may be significant that Pacific herring spawning occurs at the time of year when annual variation in tidal fluctuation is minimal (the spring equinox). Further, within this period, spawning tends to occur on neap tides when variation in diurnal tidal amplitude is minimal.

This paper presents evidence that older herring spawn earlier than younger herring, a phenomenon described for Atlantic herring and other species (Blaxter and Hunter 1982). Therefore, as herring age, their maturation schedule and spawning time must become earlier. Obviously the tendency to spawn on neap tides is not exact, but if it were, it would require that individual herring spawn about 10 d earlier, each year, of their lives. If a herring lived to 9 yr (a relatively old fish in British Columbia) and began spawning at age 3, then the same fish would have to spawn 60 d earlier in the calendar year as a 9-yr-old than it did as a 3-yr-old recruit. This range of time for the spawning season is generally too long for most spawning areas in British Columbia. Also, most single spawning locations do not have more than two or three waves of spawns. The relationship between female size and egg weight is statistically significant but quite variable. At best there seems to be a trend for larger, earlier spawning herring to have larger eggs. This indicates that as an individual herring ages, its annual spawning time would become earlier and its egg size may increase.

A consequence of a strong tidal effect on spawning initiation is that peak spawning times must change from year to year because the tidal phases change between years. Within a spawning season and in one general location there can be several spawning waves. The location of the second can be the same as the first wave or it may change by relatively small distances. For instance, within Barkley Sound, the second or later wave may be several kilometres or more from the first wave (Hay et al. 1989f). By moving about within the spawning range of a single herring stock, and perhaps spawning at different places in different years, an individual herring could attain flexibility in the timing and place of spawning. Over its lifetime, a single fish could, theoretically, produce eggs of dif-

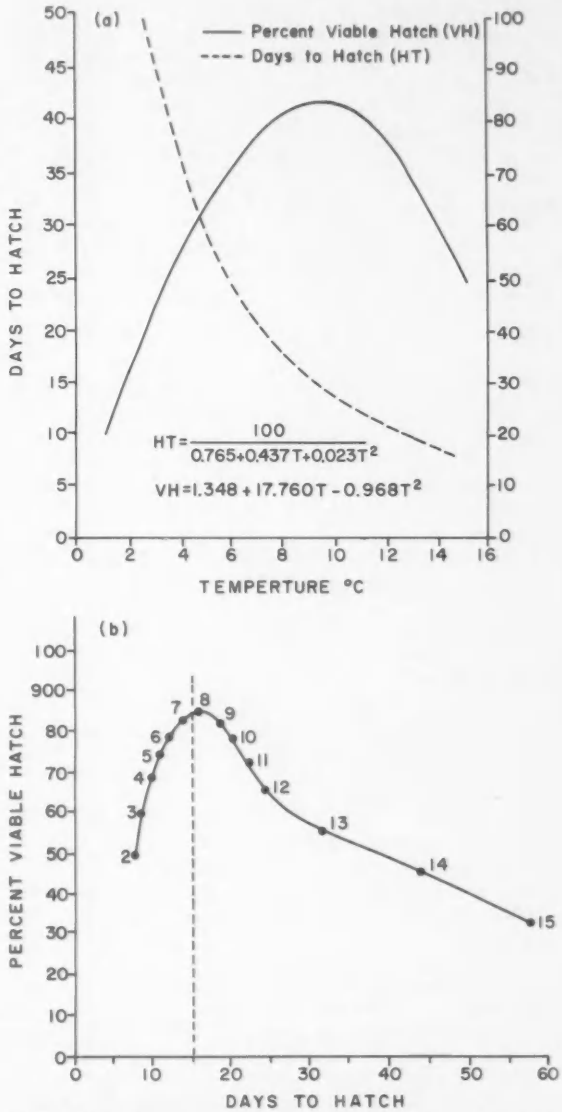

FIG. 8. Relationship between optimal hatching rate, temperature, and hatching time. (a) Days to hatch (HT), shown as a broken line, and percent viable hatch (VH), shown as a solid line, are derived from equations presented by Alderdice and Velsen (1971) with salinity held constant at 29 ppt. (b) Relationship between percent viable hatch and days to hatch, shown for temperatures between 2 and 15°C. The broken vertical line, at approximately 15 d, indicates the optimal hatching time.

ferent sizes, released at different times at different places. Results of anchor tagging studies of spawning fish confirm that some individuals spawn at different locations between years, but most herring usually spawn repeatedly within the same general vicinity (Haegele 1986). The analyses in this paper also show that spawning times within a season can be complex. This variability of spawning times appears to contradict the general conclusion that spawning times among temperate fish stocks are very regular. Some Norwegian herring stocks have mean spawning times varying by only a few days from year to year (Cushing 1969). However, *mean* spawning times of British Columbia herring also appear to be regular and the use of the mean and standard deviation to describe spawning time may give the misleading impression that spawning times are more exact than they really are. For instance, the mean date of spawning in the main spawning area of Barkley Sound (on which Fig. 4a is based) is DOY 73.03 with a standard deviation of 11.33. Although this is more variable than the Norwegian herring data (with a standard deviation of about 5–7), the mean and standard deviation of the spawning date do not reveal anything about the different spawning waves. However, the complexity of factors related to spawning time does not necessarily contradict the general conclusion that spawning times are related to the production cycle (Cushing 1972). Also, for most purposes, comparisons of mean spawning dates may be quite valid.

When spawning times are compared among different spawning areas there are some remarkable differences. For instance, some of the latest spawns on the northern British Columbia coast are in Masset on the north coast of the Queen Charlotte Islands (section 011 in Fig. 1) with a mean spawning date of June 27 (DOY 173.0). In contrast, some of the earliest spawns occur in Naden Harbour, also on the north coast of the Queen Charlotte Islands (section 112 in Fig. 1), with a mean spawning date of March 2 (DOY 61.2). Probably, these two groups represent different stocks which may be virtually reproductively isolated from each other and, perhaps, other herring stocks by their spawning times. Other examples of early and late spawners from other areas in British Columbia are listed in Hay et al. (1989a–f).

These extreme differences in spawning time must have evolved in the last 10 000 yr since glacial recession. The large spawning time differences between stocks are evidence of local and rapid selection, and such selection indicates that spawning time has significant survival value. An explanation for differences in spawning times is that the herring have spawning times that match local zooplankton production schedules, particularly the time of egg production by copepods because copepod eggs are, ovewhelmingly, the dominant food organism of larval herring (Wailes 1936). There also could be a selective advantage to the association between spawning time and tidal cycles. The tendency to spawn no neap tides, combined with the size- and age-specific differences in the maturation schedule, ensures that there is always some inherent variability of spawning time within stocks.

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Intermolt Insemination, an Alternative Mating Strategy for the American Lobster (*Homarus americanus*)

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American lobster (*Homarus americanus*) have a dual mating strategy. Although most females mate when they are newly molted, mating can occur at any molt stage if necessary. In the laboratory, virtually all uninseminated preovigerous females mate prior to spawning. Male aggression is a major factor in the success of intermolt mating and males can discriminate between immature and mature females, and between inseminated and uninseminated females. Female receptivity is affected by both ovarian stage and the presence of stored sperm, but not by molt stage. Most females become unreceptive after insemination, but their receptivity returns once the supply of stored sperm is exhausted. Intermolt mating occurs in smaller lobster that for some reason did not mate at molt, and it is an important part of the reproductive strategy of larger lobster. Lobster ≥ 120 mm carapace length frequently spawn twice without molting and often fail to store sufficient sperm to fertilize consecutive spawnings. Intermolt mating ensures these females will produce fertile second broods.

La stratégie d'accouplement du homard d'Amérique (*Homarus americanus*) est double. Bien que la plupart des femelles s'accouplent juste après la mue, l'accouplement peut avoir lieu à n'importe quel stade de la mue suivant les circonstances. En laboratoire, pratiquement toutes les femelles préovigères non inséminées s'accouplent avant de pondre. L'agression des mâles est un facteur clé dans le succès de l'accouplement inter-mue; ces derniers peuvent distinguer les femelles matures des femelles immatures et les femelles non inséminées de celles qui le sont. La réceptivité des femelles dépend du stade de développement des ovules et de la présence de sperme emmagasiné, mais pas du stade de mue. La plupart des femelles perdent leur réceptivité après insémination, mais la retrouve une fois leur réserve de sperme épuisée. Les accouplements inter-mue se produisent chez les petits homards qui, pour une raison quelconque, ne s'accouplent pas au moment de la mue et c'est aussi un élément important de la stratégie de reproduction des homards de plus grande taille. Les homards dont la carapace a une longueur égale ou supérieure à 120 mm pondent souvent deux fois entre deux mues et souvent, ils n'ont pas emmagasiné suffisamment de sperme pour fertiliser ces deux pontes successives. L'accouplement inter-mue permet à ces femelles de produire la deuxième fois une ponte fertile.

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Mating behavior in newly molted lobster, involving pair formation and cohabitation, is thought to be elicited by a suite of pheromonal, chemotatile, and visual stimuli (Atema 1986). Female *Homarus americanus* reportedly have a limited period of sexual receptivity and mating seldom occurs more than 24–48 h after the molt (Templeman 1934, 1939; Hughes and Matthiessen 1962; Aiken and Waddy 1980; Atema and Cobb 1980; Atema 1986). If females are restricted to mating on only one or 2 d every 2–4 yr, there is considerable potential for reproductive failure due to mating limitations. In areas with low population density or biased sex ratios, it is conceivable that a significant number do not mate (see Saila and Flowers 1965; Ennis 1980; Daniel et al. 1989). Since spawning occurs whether or not a female is carrying stored spermatozoa, lobster that are not mated spawn unfertilized eggs. In addition, large lobster that spawn twice within one instar do not always store enough sperm to fertilize both broods (Waddy and Aiken 1986).

The problem of lobster spawning unfertilized eggs would be mitigated if mating habits were more flexible and insemination was not restricted to stage A of the molt cycle. Although there have been reports of female lobster mating in other molt stages (Herrick 1909; Dunham and Skinner-Jacobs 1978; Dunham 1979), so-called hard-shell matings were thought to be unusual occurrences and the result of crowded laboratory conditions (Aiken and Waddy 1980; Atema and Cobb 1980; Dunham 1988). Others questioned whether mating and insemination could occur once the cuticular flaps covering the seminal receptacle harden after the molt (Nelson and Hedgecock 1971; Talbot et al. 1986).

Studies on artificial insemination revealed that female molt stage is not a constraint to insemination and fertilization (Waddy and Aiken 1985). Because of this and other puzzling aspects of mating and insemination, we reassessed sexual biology of the American lobster in a 3-yr study involving uninseminated mature female lobster throughout the molt and reproductive cycles. This paper reports the results of that study and presents a new concept of mating behavior and biology in the American lobster.

Materials and Methods

Experiments were conducted over a 3-yr period in which male and female lobster were held communally with shelters in a

2 × 2 × 1 m high tank illuminated at night with red lamps (light from 25 watt incandescent bulbs filtered through Kodak No. 1A filters) so that activity could be recorded continuously with a time-lapse video recorder. More than 120 matings of previously uninseminated females were recorded on videotape. Another 152 intermolt inseminations that were not videotaped occurred in groups housed in smaller tanks (1 × 1 × 0.3 m high).

Lobster in Experiments 1–4 were captured in the vicinity of Miminegash, Prince Edward Island. They were 75–90 mm carapace length (CL) and had previously spawned in captivity, hatched their broods and molted in isolation. As Templeman (1939) noted, sperm are not retained through the female molt, so these females were uninseminated when the experiments began. When held under local seawater temperatures at St. Andrews, lobster <120 mm CL spawn in alternate years and a year elapses between molting and spawning. The females in Experiment 5 were of Bay of Fundy stock (123–162 mm CL) and were approaching their second spawning within a single instar. Males in all experiments were mature and had previously sired progeny. Lobster in groups that were videotaped were identified by numbers etched with a dental drill on the dorsal carapace and highlighted with a white paint marker (Testors Corporation).

Densities in the experimental tanks ranged from 2–5 lobster/m^2 and the male to female ratio varied from 1:3 to 1:1. Temperature varied seasonally (0–14°C) and natural photoperiod (45°N) was provided through west-facing windows. In studies where lobster had access to shelter, a surplus of drainage tiles was used. The tiles were too small to allow cohabitation, so matings occurred in the open where they could be recorded on videotape.

During the experiments, videotapes were reviewed every 12–24 h so that it could be determined whether observed matings resulted in insemination. Spermatophores are visible when the receptacle is opened with "barraquer" forceps (Fig. 1). Females in Experiments 3–5 were retained through spawning, egg incubation, and hatching to determine the success of egg attachment and hatching from intermolt matings.

Experiment 1: Incidence of Mating in Uninseminated Preovigerous Females ($N = 47$)

Similar studies were conducted in each of 3 yr. In the first year (Group I), 18 uninseminated preovigerous females (cement gland stages ⩾ 2, Aiken and Waddy 1982) were held with five males from 30 April until 14 August. In the second year (Group 2), the experiment was repeated from 6 May until 10 August with the same males but 17 other females. In the third year (Group 3), 12 females were held with the same males from 14 May until 7 August. The entire experiment was recorded on videotape.

Experiment 2: Effect of Female Ovarian Stage on Mating Incidence ($N = 55$)

Mature females that had not mated at their previous molt were held communally with mature males from 3 May until 8 August. Thirty-seven of the females were preovigerous (cement glands 2.0 or greater in early May) while the other 18 were mature but not preovigerous (cement glands 0.5–1.0 in early summer).

Experiment 3: Effect of Seawater Temperature on Mating Incidence ($N = 110$)

Ovigerous females were maintained in isolation until their broods had hatched and the female had molted. Over the next 9 mo, the females (molt stages B–D_0) were assigned to groups according to the seasonal water temperature at the time they were held with males: Group 1, temperature 0–5°C ($N = 12$); Group 2, temperature 6–9°C ($N = 22$); Group 3, temperature 10–14°C ($N = 31$). Lobsters were held communally with males without shelter for 30 d.

In a second study, 10 females (Group 4) were held communally with three mature males from 12 February until 16

FIG. 1. Use of barraquer forceps to examine seminal receptacle for presence of sperm plug.

April (seawater temperature 0–4°C) and 35 females were held with five males (Group 5) from 25 June until 6 August (seawater temperature 10–14°C). An excess of shelter was provided. The experiment, except for Group 3, was recorded on videotape.

Experiment 4: Effect of Female Molt Stage on Mating Incidence ($N = 114$)

Mature uninseminated preovigerous females that had molted in isolation were held with males at various times following molt. Group 1 ($N = 15$), molt stage B; Group 2 ($N = 32$), molt stage C_1–C_2; Group 3 ($N = 24$), molt stage C_3; Group 4 ($N = 43$), molt stage C_4–D_0. Molt stages were determined according to Aiken (1973). The experiment was recorded on videotape.

Experiment 5. Re-insemination of Multiple Spawning Females ($N = 3$)

Three large females (123–162 mm CL) that were due to spawn a second time and were not carrying sperm were each held with a mature male (157 mm CL) for a few hours. Two of the females had used all their stored spermatozoa at the first spawning, while the third had not been inseminated at the previous molt and had spawned unfertilized eggs.

Results

Experiment 1. Incidence of Mating in Uninseminated Preovigerous Females

All females ($N = 47$) were inseminated during the course of the experiment and subsequently spawned fertilized eggs (Table 1). Despite being held together for 3.5 mo, only 10% of females in this and Experiment 4 were inseminated twice. Many of those (55%) inseminated a second time were judged on the basis of the contents of the seminal receptacle to be carrying small spermatophores.

The five males mated with similar numbers of females over the course of the experiment but almost all mating activity was confined to the years between molts and little mating occurred in the molt years (see Waddy and Aiken 1990).

Experiment 2. Effect of Female Ovarian Stage on Mating Incidence

All of the 37 preovigerous females (100%) were inseminated during the experiment, while only 11 (61%) of the mature but non-preovigerous females were inseminated (Table 1).

Experiment 3. Effect of Seawater Temperature on Mating Incidence

In groups held without shelter, matings occurred at temperatures as low as 0°C (Table 1). The success of insemination in the various groups was: Group 1 (0–5°C): nine of 12 females were inseminated (75%); Group 2 (6–9°C): 20 of 22 females were inseminated (91%); Group 3 (10–14°C): all females ($N = 31$) were inseminated (100%). The females in groups 1 and 2 that did not mate during the experiment became inseminated later in the year before they spawned.

In contrast, females provided with shelter in late winter (February – April; Group 4), had a much lower rate of insemination (three of 10; 30%) than those held without shelter (Group 1, 75% inseminated).

TABLE 1. Incidence of intermolt insemination in uninseminated mature female lobsters held communally with mature males.

	Number of females	% inseminated
Experiment 1		
30 April – 14 Aug. 1986	18	100
6 May – 10 Aug. 1987	17	100
14 May – 7 Aug. 1988	12	100
Experiment 2		
Preovigerous		
(cement gland stage 2.0–4.0)	37	100
Nonpreovigerous		
(cement gland stage 0.5–1.0)	18	61
Experiment 3		
Winter, 0–5°C; no shelter	12	75
Winter, 0–4°C; shelter	10	30
Summer, 6–9°C; no shelter	22	91
Summer, 10–14°C; no shelter	31	100
Summer, 10–14°C; shelter	35	100
Experiment 4		
Female molt stage B	15	100
Female molt stage C_1–C_2	32	100
Female molt stage C_3	24	100
Female molt stage C_4–D_0	43	100

Experiment 4. Effect of Female Molt Stage on Mating Incidence

Female molt stage had no effect on sexual receptivity. All females ($N = 114$) in molt stages B-D_0 were inseminated (Table 1).

Experiment 5. Re-insemination of Multiple Spawning Females

All three females were inseminated within a few hours of being placed in the male's tank.

Discussion

All uninseminated preovigerous females held communally with mature males were inseminated prior to spawning; no limitations to mating and insemination were found because of molt stage (stage A–D_0). Mating occurred throughout the year, even at seawater temperatures as low as 0°C (Table 1).

Stage of ovarian maturation affected incidence of mating. Males were able to distinguish between females that had mature ovaries and those that did not. Males held communally with mature preovigerous females (ovary stages 4b–6; Aiken and Waddy, 1982) and mature but non-preovigerous females (ovary stage 4a) mated with all the preovigerous females but with only 61% of the mature but non-preovigerous ones (Table 1).

Once inseminated, females were usually no longer receptive; hence the incidence of multiple insemination was relatively low (8–12%). Males rarely attempted to mate with a female that was already inseminated, even when held in confined quarters for several months.

Receptivity is restored once the sperm supply is depleted as it was in the two very large females that used all their stored spermatozoa at the first of two consecutive spawnings. In many (55%) of the females inseminated twice, the quantity of material in the seminal receptacle was judged to be less then the normal amount and did not fill the receptacle. Possibly the sec-

ond mating was induced by inadequate insemination, a phenomenon well documented in insects (Parker 1970; Walker 1980).

Although sexual receptivity in mature females peaks immediately after molting (Atema 1986), uninseminated females remain receptive until spawning, and there appeared to be a second peak in receptivity during the prespawning period. Receptivity was more dependent on ovarian stage than molt stage; apparent changes in receptivity during stages B, C, and D_0 are probably related more to ovary maturity and seasonal water temperature than to molt stage.

Since female lobster spawn whether or not they have been inseminated, intermolt mating is a mechanism that ensures fertilization of spawned eggs. Intermolt mating mitigates the effect of unsuccessful matings (Saila and Flowers 1965; Ennis 1980) and allows lobster to mate opportunistically at any time during the molt cycle. Intermolt mating becomes essential in about 20% of large females (≥ 120 mm CL) that spawn consecutively (see Waddy and Aiken 1986).

The aggressive nature of male lobster is a mjaor factor in the success of intermolt mating and consummation of the act often depends on the degree of aggression and persistence of the male (Waddy and Aiken 1990). Mating behavior in intermolt females is markedly different from that in newly molted females. When mating occurs following the female molt there is prolonged cohabitation after the female chooses her mate (Atema 1986; Cowan and Atema 1990); in intermolt mating, females are often reluctant participants and the male frequently forces the female to mate (Dunham 1988; Waddy and Aiken 1990). Many other decapods routinely mate when the female is hard-shelled and variations in mating strategy with shell hardness have been reported previously, as has recognition of both preovigerous and uninseminated females and mate selection based on the female's proximity to spawning (Hinsch 1968; Hartnoll 1969; Berry 1970; Ingle and Thomas 1974; Paul 1984; Ameyaw–Akumfi 1987; Christy 1987; Diesel 1988; Donaldson and Adams 1989).

The natural incidence of intermolt mating in the American lobster is unknown. It has even been suggested that intermolt females are rejected by males (Atema 1986) and that intermolt mating is an anomaly produced by confinement (Dunham 1988). However, this study suggests that intermolt mating is biologically necessary and a normal component of the reproductive strategy of American lobster. Apparent lack of interest in intermolt females is because most are already inseminated.

Several lines of evidence suggest that intermolt mating is a natural phenomenon: (a) eggs fertilized as the result of intermolt mating are as viable and attach as well as those resulting from postmolt mating; (b) lobster of a size that spawn more than once during an instar have not been found in the wild without stored spermatophores (S. L. Waddy and D. E. Aiken, unpubl. data); (c) incidence of insemination in most wild populations is virtually 100% (Templeman 1939; S. L. Waddy and D. E. Aiken, unpubl. data); and (d) under laboratory conditions, all uninseminated preovigerous females mate if given the opportunity. Mature females without sperm have been reported from some stocks (Krouse 1973; Ennis 1980), but when these studies were done, criteria for assessing maturity were not as well defined as they are today and the samples may have included females that would molt again prior to spawning.

Although there might appear to be a discrepancy between this study and earlier reports (Templeman 1934, 1936; Aiken and Waddy 1980; Atema and Cobb 1980; Atema 1986; Karnofsky and Price 1989), none of the other studies involved uninseminated preovigerous females. In fact, in many studies the sole criterion for selecting experimental subjects was size, and no determination was made of maturation or ovarian condition (Karnofsky and Price 1989; Cowan and Atema 1990). Only recently has it been possible to conduct a study like ours as we are now able to estimate ovarian condition and predict time of spawning in individual females (Aiken and Waddy 1982). In addition, there have been problems determining whether males are mature and recent evidence suggests that even when mature, males may have long periods when they do not breed (Aiken and Waddy 1989; Cowan and Atema 1990; Waddy and Aiken 1990).

Recognition of intermolt mating has altered traditional concepts of mating, fertility, and egg production in American lobster and has answered previously puzzling questions. Without intermolt mating, the significant reproductive potential of the very large lobster (≥ 120 mm CL) common to the Bay of Fundy, Gulf of Maine, and offshore areas would be wasted because a proportion of their second spawnings would be infertile (see Waddy and Aiken 1986). Intermolt mating also mitigates the effect of unsuccessful postmolt matings as it allows lobster to mate opportunistically at any time between molts. This is advantageous in areas where lobster exist in low density or the sex ratios are biased due to selective fishing.

In summary, the mating habits of American lobster appear to be more flexible than originally thought and are similar to those of many other decapods. While most matings occur at the female molt ("postmolt"), lobster can mate at any time between molt ("intermolt"). The receptivity of females appears to be dependent on ovarian development and the presence or absence of sperm in their seminal receptacle. Mating during the intermolt period seems to be a normal component of the reproductive strategy of American lobster; it occurs in females that were not mated at molt, in those that were inseminated with an inadequate amount of sperm, and in those that will spawn twice between molts and which have insufficient sperm to fertilize the second clutch of eggs.

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Abundance of Oil Sardine (*Sardinella longiceps*) and Upwelling on the Southwest Coast of India

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The abundance of oil sardine (*Sardinella longiceps*) on the Malabar coast is highly variable on the decadal scale. During this century there have been several periods of relatively high abundance, and several major population crashes. O-group recruitment to the fishery begins towards the end of the summer monsoon, and its success is statistically related to sea level at Cochin just prior to onset of the monsoon. At this time, sea level indicates remote forcing of upwelling, rather than the wind-driven upwelling that occurs during the monsoon. Unusually early remote-forcing appears to inhibit subsequent recruitment, perhaps through exclusion of spawning fish from the neritic zone by oxygen-deficient upwelled water.

L'abondance de la sardinelle (*Sardinella longiceps*) sur la côte de Malabar est très variable sur une période de 10 ans. Au cours des 100 dernières années, on a enregistré plusieurs périodes d'abondance relativement forte, et plusieurs effondrements de populations importantes. Le recrutement du groupe d'âge O en vue de l'exploitation commence vers la fin de la saison des moussons, et son succès est lié statistiquement au niveau de la mer à Cochin juste avant le début de la mousson. A ce moment-là, le niveau de la mer montre un forçage à distance des remontée d'eaux, plutôt que la remontée par le vent qui se produit pendant la mousson. Il semble qu'un forçage à distance inhabituellement précoce inhibe le recrutement subséquent, peut-être par l'exclusion de géniteurs de la zone néritique pauvre en oxygène.

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The variability of the fishery for oil sardine (*Sardinella longiceps*) off the upwelling coast of southwestern India was noted as long ago as 1865 when Day suggested that the stocks were so uncertain in their availability that a planned industrial expansion of the fishery would probably fail. Later, Thurston (1900) discussed the periodic disappearances of the species from the fishery as a constraint on the sardine oil trade. Since then, many have tried to predict the availability of oil sardine on the Malabar coast (Fig. 1) and to explain the relationship of sardine abundance to the catches of Indian mackerel (*Rastrelliger kanagurta*) in the same fishery. Because significant landings of anchovies (*Stolephorus* spp.) also occur in the fishery, there are some important resemblances between the pelagic fish of the Malabar coast and the pelagic fish communities of other upwelling coasts that include a sardine, an anchovy, and a mackerel (e.g. Longhurst 1971; Bakun and Parrish 1982).

Existence of information on oil sardine and mackerel landings on the Malabar coast extending back almost continuously to 1896 has already been noted (Longhurst and Pauly 1987). In this paper we examine these data critically and relate them to time-series data for selected variables of the marine environment.

Information on the relative abundance and recruitment variability of tropical fish over long periods is rare. Because O-group individuals, spawned only a few months previously, dominate the landings of oil sardine in each fishing season (e.g. Balan 1984; Raja 1969b), *Sardinella longiceps* is a particularly suitable species to investigate the relationships between stock size and environmental variability in a low-latitude pelagic clupeid fish.

Fishery for Oil Sardine and Mackerel

Consolidation of Existing Time-Series of Landings

From several sources we have consolidated a data base for seasonal landings on the Malabar coast for oil sardine for 1900–86, and for mackerel for 1925–86 (Fig. 2). The stock of *Sardinella longiceps* with which we are concerned occurs from Cape Comorin to Goa (see Fig. 1) and is probably the largest stock of this widespread Indo-Pacific species (Nair 1960). From 1956 to 1968, 86.4% of the catch (Raja 1969b; Nair 1960) was taken off Kerala (9–12°N) and 12.1% off Mysore (12–15°N). We ignore the <1% landed east of Cape Comorin, but included the India landing statistics for FAO Region 51.[1]

The original data are those assembled for both sardine and mackerel by Raja (1969b) from Nair and Chidambaram (1951) for 1925–49, and from Central Marine Fisheries Research Institute data for 1950–68, reported as tonnes (t) landed per fishing season starting in July. This series can be extended to

[1]FAO Yearbooks of fisheries statistics. Catches and landings. FAO, Rome.

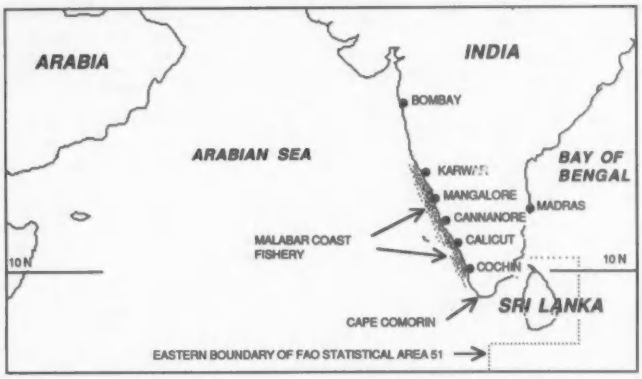


FIG. 1. Arabian Sea, to indicate the location of the principal Malabar Coast fishering towns, and the eastern extent of FAO Statistical Area 51.

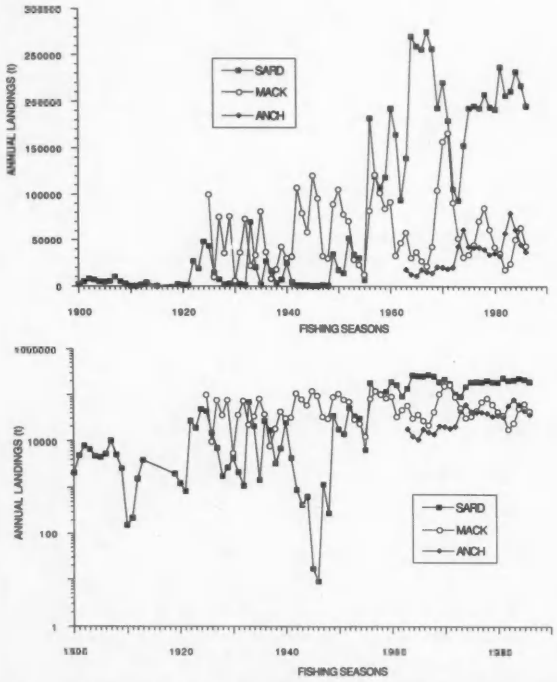


FIG. 2. Time series of the landings of oil sardine, mackerel, and anchovy on the Malabar Coast of India to show abundance changes. The lower panel is a log-normal plot to emphasise the several sardine population collapses that have occurred since 1900. Years indicated are those of the start of each fishing season.

1987 from the annual FAO Fishery Statistics for the Indian landings within FAO Region 51, which includes only the Gulf of Manaar and the Palk Straits to 12°N on the east coast of India. For the period 1954–68 the two series overlap and closely correspond. For our final data set, we used the FAO values because these probably represent the final revision of each year's statistics. The FAO data are given by calendar years, which we transformed to "fishing years" starting in July. For this transformation we used the relative proportions of landings of each species occurring in each of the two years within which each fishing season falls (e.g. landings for the season occurring in calendar years $N1$ and $N2$ = (landings in year $N1 \cdot 0.64$) + (landings in year $N2 \cdot 0.36$)). This is then quoted as "fishing season for year $N1$."

For the earlier years of the century, our extrapolation from Raja's data is much less secure. We have calculated the ratio between regional sardine landings (Raja 1969b) and regional production of "fertiliser" from oil sardine for 1925–47 (Nair and Chidambaram 1951) and then used the production of fertiliser as a proxy for sardine landings for years prior to 1925. Both data sets agree in indicating the two seasons occurring in 1925 (1924–25 = 48 000 t; 1925–26 = 43 000 t) as representing the peak of a period (1922–27) of relatively high abundance. This gives us some confidence in identifying periods of high and low abundance back to 1906. For the decade 1896–1906, we use the weight of oil sardine processed in South Kanara and Malabar (Hornell 1910) to extend our hindcast. For 1845–63, data exist for the export of sardine oil from Cochin (Day 1865) which may indicate years of good or bad fisheries on the Malabar coast. For mackerel, we have attempted no extrapolation to earlier years beyond the two basic data series (Raja 1969b; FAO catch and landing statistics[1]) for the years 1925–87, which we consolidated in the same way as for sardine.

Of course, stock abundance is not necessarily related directly to landings, but we assume this to be so for oil sardine because, as we shall discuss below, (1) reproduction occurs near the coast, so the whole reproducing population is available to the fishery, (2) fishing effort for most of the period has been artisanal, is distributed the whole length of the coast visited by oil sardine, and able to respond quickly to increasing availability, (3) the total catch of the fishery has been unregulated except for a very brief period of closure, and (4) when abundance is low on the Malabar coast, it has not been unusually high elsewhere. Finally, we note that oil sardine shoals do not perform long-distance migrations like anchovies which concentrate south of Cape Comorin in the summer monsoon season and then spread northwards along the Malabar coast in the dry season (Blindheim and Monstad 1976).

So it seems likely that oil sardine landings do represent stock abundance rather than availability or fishing effort (Banerji 1967). On the other hand, we make no such assumption for *Rastrelliger* on the Malabar coast, which seems to be the coastal portion of a widespread and less neritic stock which reproduces offshore. Because of their importance, we now examine these assumptions in detail.

Evolution of the Fishery for Oil Sardine and Mackerel

If there is a fallacy in our assumption of a relationship between stock size and landings of oil sardine, it is most likely caused by variation in fishing effort. We now examine this possibility through a review of how the fishery on the Malabar coast has evolved during the twentieth century.

Fishing gear, mostly boat and beach seines, used for oil sardine on the Malabar coast up to the late 1960's, was very similar to that described by Hornell (1910) and was developed to supply the oil and fertiliser factories established in the late nineteenth century (Nair 1960). Natural fibres for nets were replaced by artificial fibres during the 1960's, with some increase of gear efficiency, but little change in design; this increase was 10% for "Pattenkolli" (a boat seine) and 24% for all gears combined (Raja 1969a, 1969b).

This situation continued until the introduction of modern purse-seines in the mid-1970's after which the use of traditional gear progressively collapsed on the south Karnataka coast (Dhulkhed and Bhat 1985). However, neither the introduction of man-made net fibres in the decade of the 1960's nor the introduction of modern purse-seiners during the 1970's can be identified with appropriate and sustained changes in landings. We suggest, therefore, that the landings each year do, in fact, reflect relative abundance. No quotas have been set in this fishery except during 1943–47, when the fishery was closed (Raja 1969b) in response to a progressive decline to <500 t compared with landings that had previously peaked at 69 797 t. Recently, fishing by the larger seiners has been closed from June to September to protect incoming spawners (Jacob et al. 1982).

Another cogent argument is that if the decadal variation in catches of sardine had been caused by changes in fishing effort, then the catches of the other major target species should covary, yet this is clearly not the case (Fig. 2). Further, Nair and Chidambaram (1951) noted that market prices for sardines in the early years of the fishery varied between years by a factor of 13.3 according to the abundance of the fish. But most relevant is the fact that the collapse of sardine landings in the 1940's, and their growth back to very high catches during the 1950's, is not reflected in the mackerel landings, a species taken by the same boats with the same gear at least in the years of artisanal fishing. As we will discuss later, lack of covariance between sardine and mackerel is supported statistically.

Seasonal Development of the Fishery

The seasonal development of the oil sardine fishery appears to be controlled partly by the availability of the fish, and partly (at least prior to 1975) by the effect of heavy onshore monsoon winds on the small boats and canoes. During the months of strongest monsoon winds, linear banks of greenish, highly organic and mobile mud (the well-known "chakara") form inshore in many areas and provide refuge for the smaller boats (Bristow 1938; Anonymous 1980).

Each fishing season extends over two calendar years and starts in June, early in the summer monsoon, with the appearance of near-surface shoals of fish spawned the previous year (Nair 1959; Raja 1969b) at the southern end of the Malabar coast. The shoals proceed northwards (Chidambaram 1950) and at the end of the season, in February, the survivors of this year class withdraw progressively southwards together with young fish spawned and recruited to the population during the fishing season (Raja 1969b).

In the peak fishing period of September–December, catches include increasing numbers of O-group juveniles spawned by the adults which arrived in June. In January–February, surviving juveniles progressively move away, but sporadic incursions of spent-resting larger fish occur in the catches (Raja 1969b).

There are exceptions to this typical cycle; the good landings of 1965–66 were due to unusual survival of I-group fish, not to a large incoming O-group (Raja 1969b), while the 1978–79 fishery at Mangalore landed an unusually high proportion of spawning individuals in April–June (Dhulkhed and Bhat 1985). Conversely, the anomalously high landings of 1933–34 were dominated by juveniles (Devanesen and Chidambaram 1953). However, the general pattern is predictable, having the following average percentage catch distributions for quarterly periods for 1960–66 (Raja 1969b):

		Quarter			
		III	IV	I	II
Karwar	(15°N)	3.33	77.78	18.89	0.00
Mangalore	(13°N)	13.53	33.82	46.38	6.28
Cannanore	(12°N)	14.20	38.45	40.58	6.78
Calicut	(11°N)	20.83	43.82	30.48	4.87
Cochin	(10°N)	30.57	43.22	16.93	9.28
Vizhingam	(9°N)	71.43	14.29	14.29	0.00
Total		21.25	42.44	29.95	6.36

The mackerel fishery follows a similar pattern; it begins in September and declines sharply after December. During these 4 mo, about 80% of the total seasonal landings of mackerel are made.

Interannual Variability in the Fishery

Of the two species, sardine are more variable than mackerel (Fig. 3). Sardine landings have periods, mostly prior to 1950, in which landings were within the range 100 to 10 000 t and periods of variable but much higher abundance, reaching maxima of > 200 000 t. In contrast, the mackerel landings are more regular, never completely failing but with few years in which catches were unusually large or small. That the interannual variability of landings of the two species does not follow a common pattern supports our belief that variation in apparent abundance of both species is not driven by changing effort of the fishing fleet which has commonly exploited both species together.

The oil sardine landing data clearly include decadal scale trends which can be most confidently identified after 1925. The start of the "modern" data in 1925 was during the first of two periods of greater abundance that occurred during a long period of relatively modest abundance extending back to the turn of the century. If our extrapolation from oil sardine products to landings of fresh fish is valid, then periods of high abundance, comparable with the modern period, and population crashes, comparable with that of the 1940's, occurred also during the nineteenth century. We can extrapolate landings for two short periods, covering the seasons 1855–59 and 1862–63. In 1855–56, stocks were <1000 t, but in the three subsequent years, catches were much higher, reaching 70 000 t in 1859. Again between 1862 and 1863 seasons the same kind of rapid change is indicated. During the present century, as Fig. 2 shows, the dominant events have been several population crashes, two of

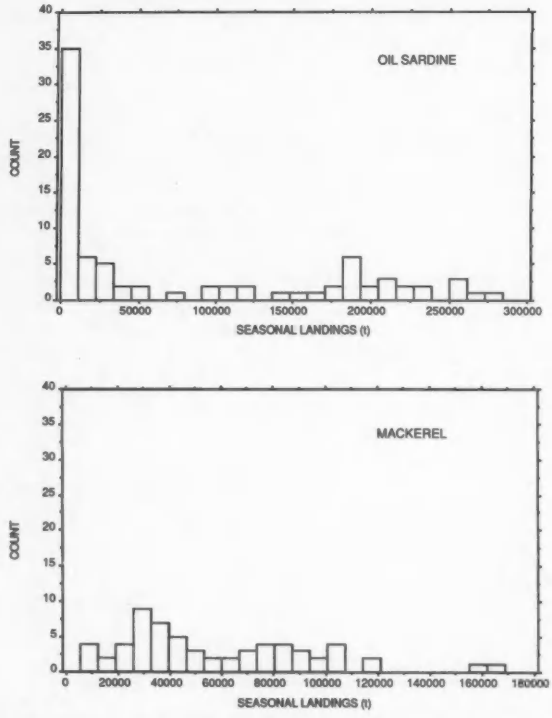


FIG. 3. Frequency distributions of landings of oil sardine and mackerel, 1939–86.

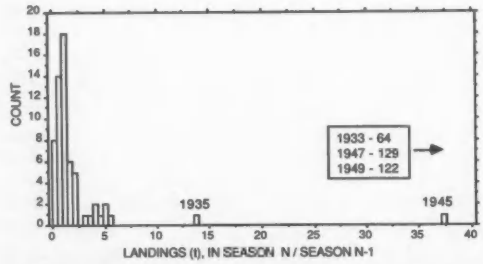


FIG. 4. Frequency distribution of a recruitment index for oil sardine given by the ratio between catches in two subsequent seasons.

which reduced landings to only about 100 t per fishing year. That in the early 1940's dominates the pattern as does the striking regrowth of the population starting in the mid-1950's which quickly reached and sustained a higher level of landings than previously observed.

Thus, there have been four major states of the sardine population during the present century: (1) periods of varying and moderate abundance prior to 1940, (2) periods of population collapse (1856–57, 1910, 1921, and especially 1941–47), (3) a period of progressive increase in population (1948–66), and (4) the modern period of fluctuating but relatively high abundance (1966–87).

We think that the disappearance of sardine in the 1940's (and perhaps earlier disappearances also) was fishery independent. It is unlikely that the fishing effort available in the late 1930's would be capable of reducing the population so radically, if a

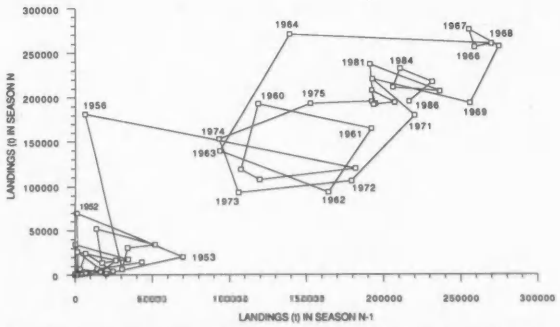


FIG. 5. Stock–recruitment analysis for the oil sardine based on the relationship between catches in subsequent fishing seasons. See the text for a full explanation of this analysis. Years indicated are those of the start of each fishing season.

few years later it was incapable of restraining population growth. Further, mackerel continued to be caught in normal abundance during the sardine crash, indicating that the fishery itself was normally active. Similarly, as we have discussed above, there is no reason to believe that the sustained trend of increasing catches during the years 1948–64 was due to increasing effort in the fishery because, again, the mackerel landings show a quasi-cyclic variability around an average catch rate which lacked major trends during the entire period for which we have data. Only for seasons immediately after 1956 and 1969 did the landings of the two species change simultaneously and with the same sign, perhaps indicating a common cause.

The numbers of juvenile fish spawned early (June–September) in the fishing season dominate each season's landings of oil sardine, so each season's catch is largely a reflection of the success of a single year class. We can thus obtain a dimensionless index of recruitment from the ratio (landings in season N/landings in season $N-1$). Figure 4 shows that this index is remarkably constant, with only occasional surges of high recruitment: the value lies between 0.25 and 2.0 for 53 fishing years, from 2 to 100 for 6 years, and exceeds 100 in only 2 years, 1947 and 1949, when the population was recovering from its lowest recorded levels.

Figure 5 shows a version of the stock–recruitment relationship which is similar to those given for other sardine stocks by Sharp et al. (1983). The cyclical pattern in the relationship between landings in successive years represents a surge of increasing recruitment, leading to an increased adult population, then followed by a decrease in recruitment and finally to a decreasing adult population. The surges in recruitment in 1962–64 and 1973–75 shown in Figure 5 correspond to periods of increasing total sardine abundance (Fig. 2). Figure 5 also shows that recruitment was stronger (abundance ratio $N/N-1$ was greater) from the 1955 season than in any other year, and it was this surge in recruitment that shifted the sardine population from a low to a high abundance regime. Comparison between this figure and a comparable representation for South African sardine (Sharp et al. 1983, their fig. 4) shows a striking difference: the cyclical pattern for South African sardine is driven down towards low stock and low recruitment by fishing pressure, while the cyclical pattern for Indian oil sardine shows a shift from low stock/low recruitment to higher levels despite fishing pressure. So the cyclical pattern of oil sardine probably reflects density dependence, rather than response to fishing pressure.

Interactions between Mackerel and Oil Sardine Landings

A density-dependent relationship between oil sardine and mackerel populations was suggested by Hornell (1910) because he observed an apparently inverse relationship between annual landings of the two species. However, we find no correlation ($r^2 = 0.001$ for 62 cases) between landings of the two species since 1925 despite the conviction of others (e.g. Raja 1969b) that an inverse relationship existed, at least over short periods. We investigated also the possibility that a relationship might exist between the abundance of the two species in subsequent seasons, but again found no correlation for either species ($r^2 = 0.002$ for lagged mackerel, $r^2 = 0.005$ for lagged sardine). If there is no simple relationship with years or between adjacent years, as Hornell supposed, are the prominent decadal-scale variations in abundance themselves caused by biological interactions? Careful inspection of Figure 2 will show that relative abundance is neither similar nor regularly opposite for the two species, although there are some periods when one species or the other is more abundant. More relevant, however, are the periods of rapidly changing abundance, and it will be seen that these may have the same, or the opposite, sign for the two species. The most fundamental change in the relationship between the two species occurred as a result of the anomalous increase in stock size of sardine during the 1950's, an event that was not reflected in a comparable phase change in mackerel abundance, which continued to oscillate between about 25 000 and about 100 000 t.

We therefore conclude that there is no obvious reason to think either that (1) changes in abundance of sardine and mackerel are driven by the same process or (2) such changes represent the consequences of competitive species replacement. Because of this conclusion, we were led to seek an explanation for the population trends of oil sardine that rests in environmental variables rather than in density-dependent relationships with other small pelagic fish potentially competing for the same resources.

Biology of *Sardinella longiceps*

We summarise biological events in the life history of *Sardinella longicips* (Fig. 6) to facilitate identification of what features of their biology are likely to be sensitive to seasonal upwelling from May to September, and so determine their subsequent abundance.

We would expect three important consequences of seasonal upwelling for oil sardine and other continental shelf fish. First, as in almost all upwelling situations, the supply of inorganic nutrients to the photic zone fuels an algal bloom, dominated by large diatoms (Subrahmanyan 1959; Subrahmanyan and Sarma 1965); such blooms may also be supplied by nutrients released from sediments resuspended by heavy onshore swell during the monsoon. Second, because the upwelled water comes from within the Arabian Sea oxygen minimum, it is deficient in oxygen. Demersal fish are excluded by it from midshelf depths and frequently suffer mass mortality against the beaches (Banse 1959). Third, wind-driven (Ekman) upwelling is associated with offshore transport of surface waters and potential advection of shelf biota to the open ocean.

The special characteristics of oil sardine biology that are relevant to life in an upwelling environment may now be briefly reviewed. Oil sardine grow rapidly, mature early, and few survive through a second year of life. It is now agreed that age at first maturity is less than 1 yr and occurs at about 150 mm. This was the opinion of Hornell and Nayadu (1924) and was confirmed by Raja (1969b), although some authors thought fish were older, and matured later (Chidambaram 1950; Nair 1953).

Some fish begin to develop gonads (males earlier than females) in April, and spawning begins during May–June, extending variably through the end of the year (Hornell 1910; Hornell and Nayadu 1924; Prabhu and Dhulked 1970; Raja 1969b). Only a few unusually old fish are active during a second spawning season (Raja 1969b). Blindheim and Monstad (1976) showed that larvae occur in a coastal band of high larval abundance extending 20–30 miles offshore, especially from 11 to 15°N, with some concentrations where the shelf is wider off the southern Malabar coast. Absolute highest abundance of larvae, moreover, is not always on the coastal side of this band.

It has been suggested that maturation may be controlled by climatic conditions (expressed as temperature and rainfall) experienced by the prespawners. Consequent changes in

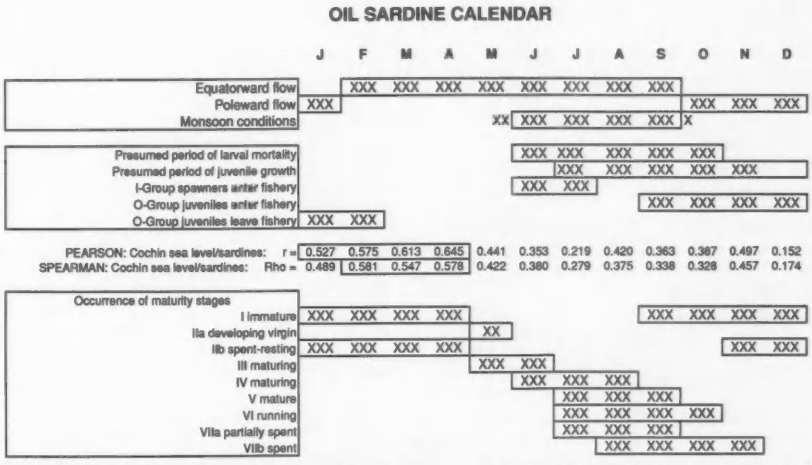

OIL SARDINE CALENDAR
J F M A M J J A S O N D
Equatorward flow
Poleward flow
Monsoon conditions
Presumed period of larval mortality
Presumed period of juvenile growth
I-Group spawners enter fishery
O-Group juveniles enter fishery
O-Group juveniles leave fishery
PEARSON: Cochin sea level/sardines: r = 0.527 0.575 0.613 0.645 0.441 0.353 0.219 0.420 0.363 0.387 0.497 0.152
SPEARMAN: Cochin sea level/sardines: Rho = 0.489 0.581 0.547 0.578 0.422 0.380 0.279 0.375 0.338 0.328 0.457 0.174
Occurrence of maturity stages
I immature
IIa developing virgin
IIb spent-resting
III maturing
IV maturing
V mature
VI running
VIIa partially spent
VIIb spent

FIG. 6. Diagrammatic calendar for the biology of the oil sardine on the Malabar coast and the major events in its environment. Monthly correlations between sea level and sardine landings in each fishing season for the period 1939–86. The periods during which each process or state occurs are indicated by blocks of X's.

fecundity would then determine the size of the O-group stock entering the subsequent fishery. In some years, the ovaries of prespawners have in fact been observed to contain a high proportion of oocytes in the process of atresia and resorption, heralding reduced fecundity (Raja 1969b, 1970, 1972).

The oil sardine is a planktivore and one of the few clupeoids in which diatoms form a significant part of the adult diet. Hornell (1910) noted that the first arrival of sardine at the coast coincides with a diatom bloom; this observation has been repeated many times since, and it has been suggested that migrations are timed to coincide with two seasonal blooms of the diatom *Fragillaria (=Nitzschia) oceanica*. The first bloom, at the start of the monsoon season, coincides with the arrival of prespawning adults, and the second, in September–October, coincides with the main fishery for juveniles (Nair 1960). Dinoflagellates and copepods are also important in the diet from October to January, together with soft organic-rich material resuspended by the seasonal dispersion of offshore chakara (Raja 1969b).

Particularly heavy blooms of *Fragillaria oceanica* were observed in 1949 and 1953, during the rebuilding of the sardine stock after the population crash of the 1940's (Nair 1953; Nair and Subrahmanyan 1955). But since there exists no time series index of diatom blooms, it has never been possible to associate diatom blooms statistically with heavy landings. In fact, some have interpreted their observations of the fishery to suggest that diatom blooms may merely act as a local attractant for sardine shoals (Raja 1969b).

Potential Relations between Water Column Stability, Offshore Transport, and Recruitment Success

Indian oil sardine are not the only clupeoids to show high variability in abundance, although strong decadal variability was unexpected in a tropical species. Stock size of clupeoids (*Clupea, Sardina, Sardinops*, and *Engraulis*) in other seas is known to vary on decadal to secular scales (Soutar and Isaacs 1974; Kondo 1980; Cushing 1981; Belvèze and Erzina 1983; Tanaka 1983; Pauly and Tsukuyama 1987).

For the clupeoid species of upwelling coasts, the sources of natural variability have been sufficiently studied for strong hypotheses to have emerged concerning causative mechanisms. Current models relate the impact of environmental conditions at the time of critical growth of larvae and postlarvae to their survival and to the success of subsequent recruitment. Two processes (both difficult to substantiate) are usually considered: (1) those which aggregate or disaggregate layers of plankton, thus providing food concentrations above or below a critical threshold (Lasker 1975, 1981), and (2) the action of wind-induced offshore drift or mesoscale eddies in transporting coastal larvae away into the open ocean (Bakun and Parrish 1982; Sinclair et al. 1985).

Integration between the different consequences of variable upwelling strength has been achieved by Cury and Roy (1989) for the cases of the Peruvian (*Sardinops sagax, Engraulis ringens*) and West African clupeoids (*Sardina pilchardus, Sardinella aurita, Sardinella maderensis*). They showed that in wind-driven upwelling situations, maximum recruitment occurs at a critical wind speed (of around 5–6 $m \cdot s^{-1}$), and at higher and lower wind speeds, recruitment progressively decreases. For remotely forced upwelling caused by the geostrophic upsloping of isopleths towards the coast, they described a relationship between recruitment and upwelling strength which is linear.

To what extent can this model be applied to oil sardine abundance on the Malabar coast? Potential considerations include the extent to which the coastal upwelling is wind driven or is due to geostrophic sloping of isopleths. Also, we must consider conditions peculiar to this coast, such as the low oxygen tension of upwelled water and the extreme nature of neritic turbidity during the monsoon season.

Seasonal Variability in the Marine Environment

The oceanography of the Malabar coast is dominated by the reversal of winds between the southwest and northeast monsoons. The southwest monsoon normally reaches the southern coast in May or June, spreads northward, and continues to blow through September. Between November and March or April, the region is under the influence of lighter, drier northeast winds. Characteristics of this climatic regime that may vary from year to year include the timing of onset and withdrawal of the southwest monsoon and the intermittency and intensity of associated wind stress and precipitation.

Ocean currents also alternate in direction in the Arabian Sea, as is well known for the Somali Current. Off the Indian coast this is demonstrated by drift estimates made by ships (Koninklijk Nederlands Meteorologisch Instituut 1952) which show equatorward flow along the Malabar coast from February through September, but poleward during the rest of the year. The equatorward flow is the eastern limb of the large-scale anticyclonic circulation of the Arabian Sea and is accompanied by upsloping of isopleths (e.g. temperature, density) towards the coast and by intrusion of subpycnocline water of low oxygen content onto the shelf (Banse 1959, 1968).

The exact mechanism which drives the upwelling has been unclear because the summer monsoon appears to blow almost directly onshore (Wooster et al. 1967). The common explanation has been that the upwelling is nothing more than the shoaling of nearshore isopleths, is a consequence of baroclinic adjustment to the anticyclonic monsoon circulation, and hence is remotely forced (Wyrtki 1973). However, Mathew (1982) noted that near the coast there is an equatorward component of wind stress through the year. He considered that this, together with the intensified summer anticyclonic circulation, was the mechanism which produces upwelling.

The magnitude of the equatorward wind stress was estimated by Shetye (1984) from large-scale climatological data to vary from 1.0 (winter) to 5.0 (summer) $\mu N \cdot cm^{-2}$. Premchand (1985) showed comparable values for 1976–78. The intensity of the wind stress varies with latitude, and Shetye and Shenoi (1988) described the monthly variation of the longshore component of wind stress along the west coast of India. At the latitude of Cochin (10°), maximum wind stress is about 3.0 $\mu N \cdot cm^{-2}$, while monthly mean values computed by Premchand et al. (1988) from three-hourly winds measured at Cochin from 1982 to 1987 range from 0.6 to 1.5 $\mu mN \cdot cm^{-2}$.

Can such a small wind stress component produce significant upwelling? Using data from Bakun (1987), the offshore transport at Cochin (represented by a conservative value) can be compared with that of other upwelling regions:

	Stress	Transport
Iberia (43°N)	2.0	20
California (39°N)	14.0	153
Morocco (28°N)	11.0	161
Cochin (10°N)	1.0	39

where stress is in microneutons per square centimetre and offshore transport is in tonnes per 100 m per second. Because Cochin lies at a low latitude, relatively weak wind stress can still produce significant offshore transport, comparable with upwelling off Iberia. We must presume that the remotely forced upsloping of the pycnocline contributes to the process, but it is difficult to distinguish from the wind-driven effect. Yet because of the apparently different response of clupeoid stocks to wind-driven and to geostrophic upwelling (see comments on Cury and Roy 1989, above), the distinction between the two effects on the Malabar coast may be critical.

To try to make this distinction, we have examined the following material: (1) maps of average mixed layer depth (Rao et al. 1989) which show the anticyclonic gyre of the Arabian Sea to be present from June through September, (2) surface drift charts (Wooster et al. 1967; see also Shetye and Shenoi 1988) which show some equatorward flow on the Indian coast as early as February–March, with the anticyclonic gyre being established in April and breaking down in October, and (3) cross-shelf sections which show subsurface distributions of temperature and dissolved oxygen, including depths of selected isopleths and the temperature gradient in the surface layer (Johannessen et al. 1987). Unfortunately, none of this material includes indices that are directly related to upwelling, e.g. vertical transport, offshore transport, or nutrient content. The first is at present not measurable, and time series of the last are not available. Offshore transport can be directly estimated from the alongshore wind stress, but such data are available only for 1982–87 (Premchand et al. 1988).

Nevertheless, we note that upsloping appears to start several months before the equatorward wind stress reaches a significant value. During this preupwelling period, equatorward surface flow has a small offshore component, and surface nutrient levels are relatively low. The surface mixed layer is relatively thin and subsurface water with low oxygen content has started to appear on the shelf (but concentrations are higher than they will be in a few months as flow intensifies). When Ekman-type upwelling begins, the equatorward surface flow intensifies and an offshore component is added, surface nutrient levels increase, the surface layer becomes still thinner, and very low (<0.5 mL·L^{-1}) oxygen concentrations characterize shelf waters below the thermocline.

The simplest model, then, for the regime on the Malabar coast seems to be that isopleth tilting in the Arabian Sea is associated with the anticyclonic gyre spun up by the southwest monsoon. Equatorward wind stress later advects the surface layer offshore leading to Ekman upwelling along the coastline.

Sea Level

Sea level is an indicator of upwelling for which long time series can be constructed for several stations, those from Cochin and Mangalore being particularly useful. We assembled the Cochin data set as follows. Data for 1939–82 were obtained from the TOGA Data Center and from the Permanent Service for Mean Sea Level (PSMSL). A separate analysis for 1979–87 was made available by Premchand et al. (1988). Unlike the TOGA and PSMSL data, Premchand's data included the small atmospheric pressure correction for Cochin. Where the two data sets overlapped, their correlation was high ($R^2 = 0.96$, $n = 42$). From these sources, a Cochin data set could be consolidated for 1939–87. A Mangalore data set from the TOGA Data Center was useful but covered only 1953–76.

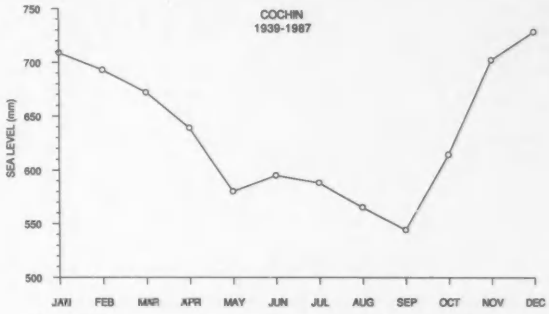


FIG. 7. Mean seasonal cycle of sea level at Cochin, 1939–87.

Average seasonal variation of the sea level at Cochin was calculated for the period 1939–87. Sea level is highest (indicating least upwelling) during the northeast monsoon, and lowest (strongest upwelling) during the southwest monsoon (Fig. 7). Between the lowest values (in May and September), there is a weak intermediate maximum during the most highly developed monsoon conditions. The seasonal cycle of sea level at Mangalore is similar except that the intermediate summer maximum is stronger, to the extent that sea level is higher during July than any other month.

Sea level is a function of (1) steric effects of the density of the water column, so that rainfall and runoff during the summer monsoon would be expected to raise sea level which may partially explain the observed maximum in July at Mangalore, (2) intensity of alongshore flow, so that low sea level is associated with strong equatorward flow, and (3) vertical circulation, so that low sea level is associated with upwelling. Relative values are also affected by (4) vertical displacement of the coastal land level.

Some information on steric changes can be gained from oceanographic data for the Malabar coast. During 1971–75, short oceanographic sections were made at intervals of 1–2 mo (Johannessen et al. 1987). One of us (W.S.W.) determined several upwelling indices from these data for 25–28 occupations of a station off Cochin in about 50 m of water; these were ΔT (difference in temperatures at 0 and 50 m), Z_{25} (depth of the 25° isotherm), and $Z_{3.0}$ (depth of the 3.0 mL·L^{-1} isopleth of dissolved oxygen). All indices were highly correlated with sea level (especially ΔT, $r = 0.81$, $p < 0.001$) for the month of the observations; however, it is necessary to be cautious in dealing with such analyses when some degree of autocorrelation presumably exists in the data sets, and we should not accept indications of high levels of correlation uncritically.

A similar test was made using Mangalore sea level data and oceanographic data taken off Kasaragod, about 14 nautical miles to the south. Here there were only 14 cases for comparison, and only the correlation with ΔT was significant ($r = 0.55$, $p = 0.05$). These data, especially those from Cochin, support the belief that the changes in sea level result primarily from steric changes associated with alterations in the pycnocline depth over the shelf; these in turn arise from upwelling, whether remotely or locally forced.

There is also information on the addition of freshwater at the surface. While the minima in the sea level cycle must indicate upwelling, the intermediate July maximum has been attributed to runoff associated with monsoon rainfall (Banse 1968). This explanation can be tested for the years 1939–81 by correlating Kerala rainfall for the monsoon season with sea level for the

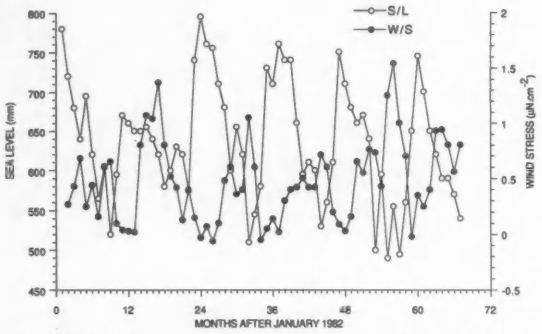


FIG. 8. Serial data for wind stress and sea level at Cochin for the years 1982–87 (data from Premchand et al. 1988).

months of June, July, and August. The correlation is significant only in July ($r = 0.36$, $p < 0.02$) and is not significantly improved if the sea level rise from June to July is substituted for July sea level.

Wind Stress

During the period (1979–87) for which Premchand et al. (1988) reported sea level and wind stress data, these are inversely correlated ($r = 0.62$, $p < 0.001$). Seasonally, wind stress variability is the inverse of the seasonal sea level curve, with a spring maximum in April–May followed by decreased equatorward wind stress in June and July contributing to the secondary sea level maximum then (Fig. 8). Peak equatorward wind stress in summer occurs in August, while lowest sea level lags by approximately 1 mo.

Interannual Variability of the Monsoon System

Because of the recurrence of droughts and famines on the Indian subcontinent, an understanding of the variability of the southwest monsoon and its associated rainfall has preoccupied meteorologists in India for more than a century. We examined the possibility that the historical records of rainfall, as an index of monsoon timing and intensity, might usefully hindcast upwelling. Wind would also have been useful, but unfortunately, a suitable time series of data could not be located. However, our most direct data to hindcast upwelling is sea level from three stations: Cochin, Mangalore, and Bombay; unfortunately, these data do not extend so far back as rainfall records.

Rainfall

The longest time series of meteorological observations related to intensity of the summer monsoon is for rain gauge data initiated nationally by the Indian Meteorological Department in 1841. Many analyses of these have been made, usually pooling the principal months of the monsoon season (July–September) to obtain annual values, or departure from long-term means. Shukla (1987) compiled regional rainfall anomalies for the years 1901–86; one of his regions (Division 31) is the west coast of Kerala (Fig. 9A). An associated measure is the date of monsoon onset, indicated by rainfall in Kerala; Shukla (1987) recorded these dates for the years 1901–78 (Fig. 9B), showing a trend toward earlier onset in the first quarter of the century. As we shall discuss later, these data are available for correlation

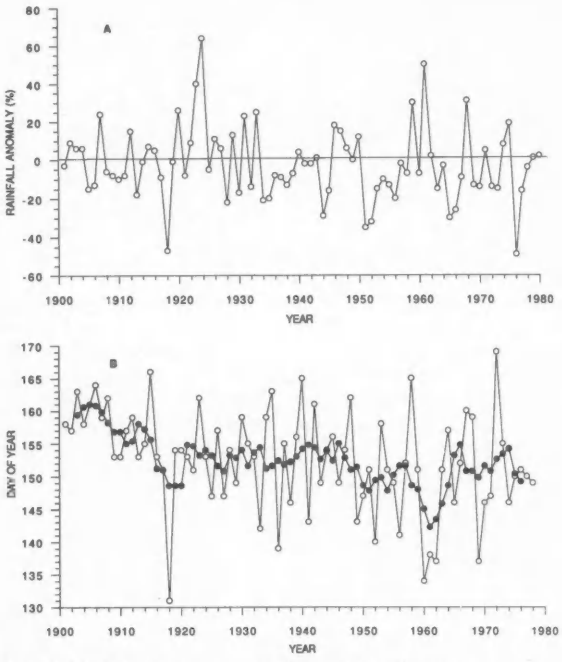


FIG. 9. Variation in monsoon conditions during the present century. (A) Serial data for the seasonal (June–September) rainfall anomalies as percentage departure from mean for the west coast of Kerala (Shukla 1987); (B) day of the year of the onset of the monsoon on the Malabar coast (day 145 is 25 May, day 160 is 9 June).

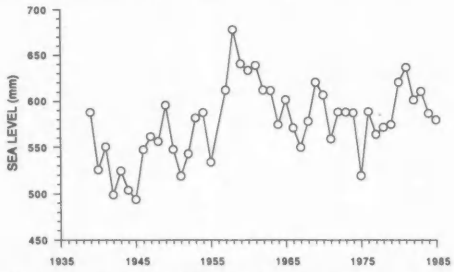
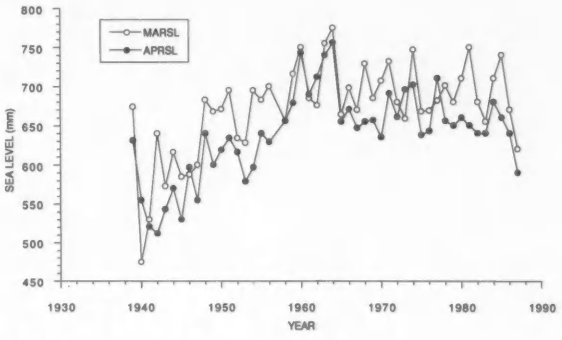


FIG. 10. Sea level at Cochin during the monsoon months May–September for the years 1939–86.

with oil sardine abundance. Sustained decadal trends or shifts between regimes occurred in neither of these sets of data.

Sea Level

However, during the 48 yr for which we have sea level data at Cochin, there have clearly been decadal trends that require interpretation. We examined these for seasons and individual months. Figure 10 shows Cochin sea level for 1939–87 during the monsoon period each year (May–September), while Figure 11 shows mean sea level for individual months for 1939–87. Remotely forced and wind-driven upwelling seem to be somewhat independent because the strongest decadal trends occur in the April data and to a lesser extent in November, while for the monsoon months (exemplified by June), decadal trends are less conspicuous. The Cochin data show a trend of increasing April sea levels from about 1942, reaching its maximum around 1960,



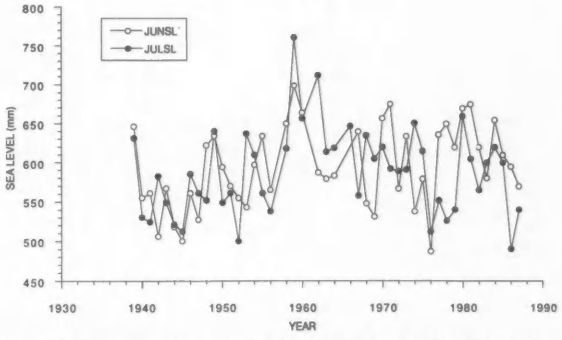


FIG. 11. Average sea level at Cochin, 1939–86, for individual months prior to and during the period of the southwest summer monsoon.

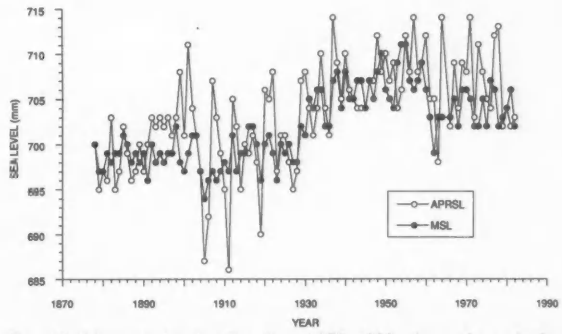


FIG. 12. Mean sea level at Bombay, 1878–1982, shown for a single month just prior to the monsoon season and as an annual mean.

after which a slightly lower level was sustained until the end of the data set. Cochin and Mangalore sea levels differ in detail although sea level anomalies correlate reasonably in May and June in the two data sets (May, $r = 0.72$, $p < 0.001$).

Somewhat similar trends occur in the longer record for Bombay (Fig. 12) which shows rising sea levels from about 1930 until the mid-1950's, with a subsequent minor lowering of sea level, although this was initiated earlier than at Cochin. It is unlikely that the occurrence of highest sea levels along the west coast in the mid- to late 1950's at two such distant stations could be a matter of chance.

The interannual variability in sea level also includes the long-term isostatic trends in sea level which can result from changing elevation of coastal lands or from changing volume of ocean water. Emery and Aubrey (1989) examined isostatic trends in

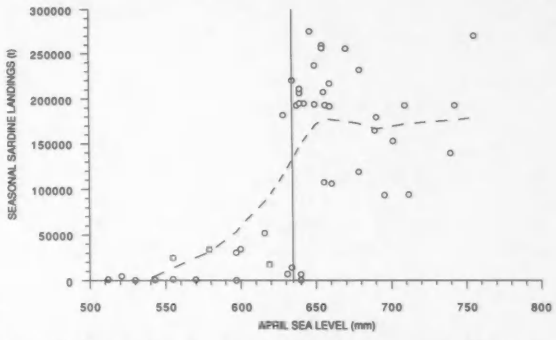


FIG. 13. Regression of seasonal sardine landings at Cochin against local sea level during April of the year in which the season commences, 1939–86. Broken line was fitted by locally weighted least squares, and the vertical line suggests the existence of two regimes.

sea level on the Indian coast and identified a rising trend at Cochin of about 2 mm·yr^{-1}, while at Mangalore sea level was falling about 1.3 mm·yr^{-1}.

Our data show similar results (1.9 mm·yr^{-1} increase at Cochin and 2.0 mm·yr^{-1} decrease at Mangalore). However, the Cochin trend is strongly influenced by low values before 1950 (see below); data for these years are not available for Mangalore. If the same period, 1953–76, is used for the comparison, Cochin sea level is also found to be decreasing only 0.3 mm·yr^{-1}. Premchand (1985) found a much stronger decrease at Cochin during 1979–87, 8.7 mm·yr^{-1}. The cause of these trends is unknown. However, existence of a secular trend in isostatic sea level, whatever its cause, should not obscure the higher frequency variability in which we are interested.

Relations between Catch and Environmental Indices

We are now in a position to compare the ecology of Malabar coast oil sardine with relationships between upwelling intensity and sardine recruitment which have been modelled for other upwelling regions.

We have compared variations in oil sardine abundance sea level as an upwelling index at Cochin, and monsoon rainfall, because it has already been suggested as an indicator of recruitment success, and also because of the length of the data series.

Relations with Sea Level

Simple comparison of the trends in Cochin sea level (Fig. 10, 11) and oil sardine catches (Fig. 2) shows that the period of unusually low sea level values during the 1940's coincided with the lowest recorded abundance of sardine. After the very early and strong April upwelling of 1942, there was a sustained trend to reduced (and probably later) April upwelling until 1964, approximately when the sardine population peaked. Subsequently, March–April sea level remained relatively high, as did catch, until the end of the available data series in 1987. Landings of oil sardine in each fishing season are significantly correlated with mean sea level in the year in which the season starts except for December. Correlation is strongest (Fig. 6) in the premonsoon months February–May, peaking in April ($r = 0.645$, $p = 0.001$), and weakest or not significant in the post-monsoon months (e.g. December, $r = 0.152$).

During the period 1939–86, above-average sea level in March–April (>650 mm) is associated with landings >100 000 t; while below-average sea level (<600 mm) is associated with landings <50 000 t. There may be some falling off of landings at the highest sea levels as can be seen from the April regression (Fig. 13). To determine if this correlation was caused solely by the low sea level and anomalously low catches of the 1940's, the relationship was also examined omitting the 1939–49 data. Correlations were weaker in all months, but the same pattern was maintained: strongest in March and April (r = 0.377 and 0.332) and weakest in the second half of the year.

Thus, we conclude that events during the early part of the year, especially March–April, have the greatest influence on the size of the sardine stock in the subsequent fishing season. The occurrence of low sea level in March–April implies either early wind-driven upwelling or early intensification of the equatorward coastal current and consequent upsloping of isopycnals.

Relations with Rainfall

Rainfall not only hindcasts monsoon timing and intensity, but Raja (1972) thought that the success of sardine recruitment could be predicted by rainfall during the weeks of spawning. He found a significant correlation between rainfall then and subsequent abundance of O-group sardine for the period 1960–69. Our analysis, using seasonal rainfall and landings for a much longer period, does not confirm this finding.

For the years 1939–81, correlation between rainfall and sardine catch is not significant, and rainfall in Kerala State (Fig. 9) has no decadal-scale trends during the 1940's and 1950's. Comparison with Fig. 2 reveals that periods of unusually high or low sardine abundance are unrelated to periods of unusually high or low rainfall.

However, the date on which the monsoon reaches Kerala does display an interesting pattern of variation. Because the monsoon goes on to spread northward from Kerala, the onset date there has more than local significance. There have been two periods when the monsoon arrived progressively earlier, one during the first decades of the century and the other, although showing a less consistent trend, from 1945 to about 1960 (Fig. 9). We note that major periods of increasing sardine abundance occurred only during periods when monsoon onset was trending towards earlier, rather than later, onset dates.

Thus, during the low catch, low sea level period in the early 1940's, monsoon onset was relatively late (1942–48 = June 4). As catch increased, and April sea level progressively rose, the monsoon arrived progressively earlier (1949–55 = May 28). As catch increased and April sea level rose even further, monsoon onset was still earlier (1956–62 = May 25).

A similar progression in the date of monsoon onset occurred at the beginning of the century (1903–09 = June 9; 1917–23 = May 31). As we have discussed above, following a period of apparently low oil sardine catches, annual landings increased in the early 1920's, reaching 15 000 to 50 000 t.

Although a long time series of wind data useful as a direct index of monsoon intensity is not available, Murty and Edelman (1971) used atmospheric pressure differences (Cochin–Bombay and Cochin–Madras) as an index of monsoon intensity. For 1929–55, they did find significant correlations between sardine catches (landings) and pressure differences, with decreasing catches up to an intermediate value and increasing catches at higher values. Most of their data came from years of low sardine abundance, and only the last years of the record (1948–55) showed increasing catches with increasing pressure difference. This observation is difficult to reconcile with our finding of generally rising sea level during this period and illustrates the difficulty of correlation studies using relatively short time series, as Koslow (1984) recently emphasised.

Discussion — Environmental or Competitive Forcing of Population Size?

The fisheries literature is replete with correlations between stock size and environmental conditions (and between stock sizes of sympatric species) that have stood for a period and then apparently failed, but Skud (1983) discussed how such failures may be more apparent than real. He suggested that any simple correlation may fail eventually because the species involved may have different responses to the same environmental signal according to whether they are dominant or subordinate to a competing species. He cited several examples where an apparently failed relationship may be explained by a more careful examination of how, and why, the species reactions to their environment have altered. The small pelagic fish of the upwelling Malabar coast offer us another, and so far relatively neglected, upwelling ecosystem where this principle can be investigated.

There are several possible ways in which the abundance of young oil sardine recruiting to the Malabar coast fishery at the end of the southwest monsoon might be determined, but any model has to explain a statistical relationship between recruitment and environmental conditions which is strongest several months (March–April) prior to the critical time of larval growth (July–October); we think it is no coincidence that this is the life history period often assumed to determine subsequent recruitment.

Oil sardine larval ecology is typical for tropical clupeoids; after occlusion from planktonic eggs, development is rapid, yolk sac absorption being completed by day 3 (Nair 1960). Growth of postlarvae and juveniles is rapid, reaching 100 mm within 3 mo. Maturing fish, spawned the previous year, pass from stages I–II (immature, developing-virgin, or spent-resting) to III (maturing) during the period May–June (Raja 1972) so larvae are rarely occluded before July and must pass through their critical first-feeding period (Hunter 1981; Lasker 1975, 1981) during July–October. The earliest-spawned individuals begin recruiting to the fishery towards the end of this period.

It is therefore in the very earliest stages of maturation that oil sardine's encounter environmental conditions that are associated with later abundance of recruits. There are two possible families of explanation for this observation: (1) April sea level signals the setting-up of oceanographic conditions which will be manifest several months later as a coastal regime inimical either to the entry of spawning shoals to the coastal waters or to the survival of larvae at the time of their critical first-feeding or, alternatively, (2) unusually early remotely forced equatorward flow, with upsloping of isopleths towards the coast, depresses the prespawning maturation process so that subsequent spawning is relatively poor.

The first kind of explanation might be supported by the invasion of the shelf with water of extremely low oxygen content which occurs during the Malabar coast upwelling (see above), and we should consider if this might prevent sardine from approaching the coast to spawn. Certainly, oxygen-deficient

water is not solely a near-bottom feature and rises to within 10 m of the surface off Cochin towards the end of the monsoon period (Banse 1968; Shah 1973; Johannessen et al. 1987). Perhaps sardine appear at the surface near the coast during the upwelling season simply to avoid oxygen-deficient water: by doing this, sardine may be able to tolerate the arrival of upwelled water in most years, while demersal fish are constrained to go seawards or crowd up to the beach, as described by Banse. It is likely that in years of very strong upwelling (as during the 1940's period of low sea level), oxygen-deficient water may even break the surface. In such a case, might sardine be held offshore and so excluded from spawning and juvenile nursery grounds?

Correlation between landings of both mackerel ($r = 0.101$ to -0.129) and anchovy ($r = 0.275$ to -0.480) and monthly sea level is much weaker than for oil sardine and shows no seasonal pattern, although these are also shoaling species taken in the same fishery during approximately the same season as oil sardine. An explanation for the different relationship that exists between upwelling and oil sardine and the other pelagic species must be sought in differences in their ecology (and probably their reproductive ecology).

In fact, such a difference comes easily to hand: the reproductive ecology (maturation and larval survival included) of the three species is quite different. Mackerel reproduce intermittently throughout the year, and the UNDP Pelagic Fisheries Project surveys (Blindheim and Monstad 1976) showed that larval mackerel occur in all months. Although the species is thoroughly familiar to fisheries biologists on the Malabar coast, the lack of a single, major spawning event has led to larval mackerel morphology and seasonality being relatively unknown. For *Stolephorus* spp., the situation is different again: during the southwest monsoon, the stock tends to congregate south of the region of the oil sardine fishery around Cape Comorin and then migrates north along the whole Malabar coast during the dry season (UNDP 1976). For these reasons, we should not expect anchovy and mackerel spawning to be influenced by monsoon conditions and upwelling in the same manner as oil sardine, which is precisely what we see in the statistical relationships.

Each of the three species may respond differently to low sea level (indicating upwelling) during March–April. Only for sardine would this interfere with successful spawning, in some way to be explained. For mackerel, extended spawning throughout most of the year perhaps ensures that successful recruitment elsewhere can make up for lost potential during the early monsoon. The response of anchovy is perhaps simply to withdraw earlier than usual southwards around Cape Comorin.

It is also possible that remotely forced isopleth upsloping may interact with wind-driven coastal upwelling to vary offshore transport over the continental shelf or the incidence of mesoscale eddies at the edge of the continental shelf capable of advecting shelf water offshore. Unfortunately, we do not have the serial data of coastal wind stress required to investigate this possibility. If the degree of isopleth upsloping towards the coast, remotely forced by the circulation of the Arabian Sea, were to reinforce the offshore transport due to wind-driven upwelling, then another potential link between April sea level and subsequent recruitment would exist: in years when this occurred, it might be thought that unusually heavy loss of larvae to the open ocean would occur.

Beyond these potential mechanisms based on oxygen deficiency and offshore advection, it remains to explore, then, the second way in which sardine recruitment failure might be linked to unusually early, remotely forced upwelling. How could the maturation or larval survival processes be influenced by low sea level prior to the onset of the monsoon and several months before wind-driven upwelling occurs at the coast?

Because of suggestions of a relationship between variable fecundity and later recruitment, we first examine this possibility. Raja (1972) showed that oil sardine fecundity varied from about 30×10^3 to 45×10^3 eggs for a "standard" 46-g fish between each of six seasons (1960–65) which is, in fact, a relatively low variance for this value in clupeoids (D. Iles, Biological Station, St. Andrews, N.B. E0G 2X0 Canada, pers. comm.). It was also found (Raja 1970) that during the spawning season, as ovaries progress from stage IV to V, a variable percentage of fish each year showed vascular hypertrophy and atresia of ovarian follicles: over a small period (1960–65) he believed that there was a relationshp between the percentage of fish with degenerating ovaries and subsequent recruitment to the fishery.

The biological calendar (Fig. 6) for oil sardine, however, shows that IV–V maturation occurs when correlation between upwelling and recruitment is relatively low. If there is a direct effect of upwelling strength, as indicated by sea level, in March and April, it must operate on fish in a much earlier stage of maturation: in fact, it must operate prior to the change from stages I (immature) and IIb (spent-resting) to stages IIa (developing virgin) and III (maturing). Thus, it would be difficult to support a hypothesis that unusually early remotely forced sloping for isopleths has a direct effect on maturation leading several months later to reproductive failure. However, an indirect effect would be possible during prematuration fattening. Maturation in fish is usually supported by the mobilisation of lipid reserves, accumulated during a period of growth and feeding, into gonadial growth and maturation. To the extent that the accumulation of lipid reserves is constrained, subsequent spawning will be suboptimal. However, there is no evidence for the oil sardine that this process occurs critically in the months just prior to maturation. In fact, the lipid content of oil sardine begins to increase (Sen and Chaluvaiah 1968) about the end of the summer monsoon period in September and October and continues relatively high until February–March, after which a decline leads to minimal lipid values in June and July, when spawning is most intense.

The suggestion that fattening of prematuring fish is constrained early in the year when sea level is low is also somewhat counterintuitive. In fact, one might have predicted the opposite effect, because we must assume (in the absence of serial data on phytoplankton abundance) that even remotely forced upsloping of isopleths will tend to result in an enhanced and earlier diatom bloom and (other things being equal) in enhanced feeding conditions for the prematuring fish.

That this does not happen reinforces our earlier suggestion that any invasion of the shelf by water with low oxygen tension would tend to exclude oil sardine (presumably along with other pelagic as well as demersal species) from the coastal region, where diatom blooms are presumably most intense. In such an event, it is oil sardine, rather than mackerel, whose spawning strategy will place at risk that year's recruitment. We suggest that this is the most likely explanation of the statistical relationship between sardine recruitment failure and unusually early remotely forced upwelling.

Because each of the three species we have analysed responds differently to monsoon conditions and upwelling, we have no

reason to believe that the changes in abundances of the pelagic species on the Malabar coast can be ascribed to interspecific competition and consequent alterations in specific dominance: competition for resources there must be, obviously, but we believe it cannot yet be demonstrated to be a factor in changing abundances of oil sardine and mackerel.

If these speculations prove to be correct, then the Malabar coast oil sardine, which is one of the major pelagic stocks of the world, has sufficiently different critical relations with its biotic environment that general models applicable to other major sardine stocks may not be relevant to it. The three major stocks of the Pacific *Sardinops sagax* (off Japan, California, and Peru) appear to have increased in size simultaneously in the 1970's after a simultaneous population collapse in the 1940's (Kawasaki 1983). This common pattern is not reflected in the oil sardine, whose changes in stock size occurred quite differently in time, although they were of the same scale, both relatively and absolutely, as those in the stocks of *Sardinops sagax*. This difference in timing suggests that a unique mechanism drives oil sardine abundance, probably related to the unique character of the water upwelled onto the west coast of India. It is unlikely that the recruitment models for the *Engraulis* stocks of California and Peru will be applicable to the Indian stocks of oil sardine. The similarities between our oil sardine model and the Pacific stocks of *Sardinops* seem to be that periods of population growth decline may be associated with major changes in the physical environment.

If oil sardine recruitment is, in fact, driven by basin-scale processes in the Arabian Sea, then prediction for the fishery may become practicable at the strategic, if not the tactical, level as prediction of ocean-basin scale circulation itself becomes practicable in the coming decades. It is also likely that the availability of yet another example of the highly abundant, highly variable clupeid stocks that occur worldwide may assist us in the analysis of these enigmatic fish and the management of their ephemeral fisheries.

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Studies on Mitigation of the Effects of Acidic Precipitation on Adult Atlantic Salmon (*Salmo salar*) — Introduction

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(JA617)

Many of the poorly buffered river systems in southwestern Nova Scotia are now devoid of Atlantic salmon (*Salmo salar*) populations due to their acidification by atmospheric deposition. The high economic value of this species and the uniqueness of each riverine stock has resulted in programs designed to protect many of these stocks. Mitigation attempts have focussed on reducing atmospheric inputs of acidifying substances and restoring watershed pH through use of limestone.

Freeman et al. (1983) reported abnormally low blood plasma concentrations of androgenic steroids (testosterone and 11-ketotestosterone) in wild male salmon captured in the acidic Westfield River in southwestern Nova Scotia; normal levels returned when fish were held for a short time in more neutral water. These results led us to study adult salmon, held in cages in the Westfield River (or the nearby Medway River as a control) over the period of their sexual maturation (about 3 mo — complete experimental conditions are given in Sangalang et al. 1990). We investigated the efficacy of (1) dietary modification (in this case the addition of NaCl to replace blood electrolytes lost by fish when held in acidic water) and (2) restoration of more neutral conditions by passing the acidic river water through a bed of calcium carbonate (marble chips) upstream of the holding facility. Following manual spawning, the eggs were held to determine viability and hatchability. Immediately post-spawning a number of investigators came together on site to study these fish using a variety of techniques. The strength of this experiment lies in the cooperation of these investigators which generated a large amount of data on the sub-lethal effects of acid precipitation, all of which have been obtained from the same animals. Compared to controls, fish held in acidic water grew poorly, produced fewer, smaller, less viable eggs, demonstrated abnormal sex and stress hormonal metabolism, and experienced a high post-spawning mortality.

Sangalang et al. (1990) report that dietary salt resulted in higher peak levels of blood hormones, improved growth and fecundity with lower egg mortality, and a higher incidence of spawning fish. However, the overall performance was still below that of the control fish and the hormonal metabolic results indicated that the fish were still experiencing stress. The reproductive performance of the fish held in limed water was almost as good as controls. Liming, however, resulted in early peaking of blood hormonal levels of androgens and of 17α, 20β-dihydroxy-4-pregnen-3-one, a follicular mediator of gonadotropin and necessary for final oocyte maturation and spermiation. Fish from the limed water still showed hormonal metabolic patterns characteristic of stressed fish.

Brown et al. (1990) found that exposure to acidic water reduced plasma osmolality, 3,5,3′-triiodo-L-thyronine (T_3), Na^+, Cl^-, and Ca^{2+} (in females only), while levels of K^+, glucose, and an unidentified osmotic fraction were elevated compared to control fish. Primary lamellar epithelial hyperplasia was present in the gill tissue of the acidic fish. Fish handling problems prevented a full evaluation of the effects of dietary salt, although the fish demonstrated plasma osmolality and ion levels closer to those of the control fish. In the case of liming, plasma Na^+ and Cl^- levels and epithelial thickness of primary gill lamellae were similar to controls. Plasma glucose, T_3, and female Ca^{2+} levels were not restored to normal by liming.

Majewski et al. (1990) found that exposure of fish to acidic water resulted in a depletion of renal and hepatic acid-soluble thiol (AST) and of renal ascorbic acid. Neither dietary salt nor liming maintained these reserves at normal levels. Observed declines in bone Ca and P and muscle Na and K were coincided with elevated liver glycogen, suggesting that gluconeogenesis is an adaptive mechanism associated with exposure to acid water. Liming restored muscle Na and K levels but not bone Ca and P levels.

Jagoe and Haines (1990) report that salmon held in acidic water had more numerous, larger, and more spherical chloride cells and more mucous cells in their gills than normal. The difference in chloride cell number was due to increased cell numbers on the primary lamellar epithelium. Liming was only partially effective in preventing changes in gill histology.

Overall, the results of these cooperative studies have shown that neither dietary salt nor liming were completely effective at maintaining normal, sexually maturing adult Atlantic salmon. The inability of pH restoration with limestone to mitigate the observed effects suggested that acid precipitation results in other toxic materials being present in acidified poorly buffered river systems, or that limestone treatment itself has a detrimental effect, either directly or through the inability of the experimental system to avoid small magnitude but sudden pH changes, such as that resulting from the daily raking. Long-term protection of salmon stocks in poorly buffered river systems using water treatment methodologies is seen to require better chemical mitigation agents coupled with careful control to avoid sudden changes in water parameters such as pH.

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Effects of Diet or Liming on Steroid Hormone Metabolism and Reproduction in Atlantic Salmon (*Salmo salar*) Held in an Acidic River

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Attempts to avert the impacts of an acidic river environment on Atlantic salmon (*Salmo salar*) were carried out in 1985 and 1986. Salmon were held in the Westfield River (pH 4.7–5.2) and the nearby Medway River (pH 5.3–5.6) during their sexual maturation. A diet containing 3% NaCl was fed to the Westfield salmon in 1985. Marble chips were used to elevate the pH of Westfield River water in 1986. Fish fed the salt diet had higher peak levels of plasma sex hormones, higher fecundity, greater incidence of spawners, lower egg mortality, and less weight loss than fish fed a commercial trout diet. The reproductive performance of fish held in limed water (pH 5.1–5.9) almost attained the level observed in the Medway (control) fish. Limestone treatment stimulated early peaking of blood androgen levels, testosterone, and 11-ketotestosterone in Westfield males, and 17α, 20β-dihydroxy-4-pregnen-3-one, a follicular mediator of gonadotropin, in a few Westfield females. The head kidneys produced more cortisol and corticosterone in all Westfield fish in both years compared to Medway fish. The results suggest that neither dietary salt nor liming completely prevented the decline of reproductive performance and the alteration of steroid hormone metabolism in salmon.

Des tentatives ont été entreprises en 1985 et 1986 afin de prévenir les incidences d'un environnement acide sur le saumon de l'Atlantique (*Salmo salar*). Des saumons ont été placés dans la rivière Westfield (pH 4,7–5,2) et dans la rivière Medway avoisinante (pH 5,3–5,6) pendant leur phase de maturation sexuelle. En 1985, le régime alimentaire des saumons de la rivière Westfield contenait 3% de NaCl. En 1986, des morceaux de marbre ont été utilisés pour élever le pH de l'eau de la rivière Westfield. Chez les poissons dont le régime alimentaire contenait du sel, les taux maximum d'hormones sexuelles plasmatiques étaient plus élevés, la fécondité plus grande, le nombre de géniteurs plus élevé, la mortalité des oeufs plus faible, et la perte de poids moindre que chez les poissons recevant un régime commercial pour les truites. La reproduction des poissons placés dans de l'eau chaulée (pH 5,1–5,9) atteignait presque le taux enregistré chez les poissons de la rivière Medway (témoin). Le traitement à la chaux a favorisé l'atteinte d'une teneur maximale précoce en androgènes dans le sang, de testostérone et de 11-kétotestostérone chez les mâles de la rivière Westfield, et de la 17α, 20β-dihydroxy-4-pregnen-3-one, médiateur folliculaire de la gonadotropine, chez quelques femelles de la rivière Westfield. Le pronéphros a produit plus de cortisol et de corticostérone chez tous les poissons de la rivière Westfield au cours des deux années comparativement aux poissons de la rivière Medway. D'après les résultats, ni le régime alimentaire contenant du sel ni le chaulage n'ont complètement éliminé la baisse de la reproduction et l'altération du métabolisme des hormones stéroïdes chez le saumon.

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Many poorly buffered river systems in southwestern Nova Scotia are now devoid of spawning populations of Atlantic salmon (*Salmo salar*) due to acidification (Farmer et al. 1980; Watt et al. 1983) and there are many others where salmon stocks continue to decline as the acidity approaches critical levels. The Westfield River is one of the latter rivers. It has been the site of our investigations into the effects of an acidic river environment on the reproductive physiology of the Atlantic salmon since 1981 when Freeman et al. (1983) found abnormally low levels of the androgens, testosterone (T) and 11-ketotestosterone (11-KT), in wild, sexually mature, male salmon caught in the Westfield River (pH 4.7) compared to normal levels in similar fish caught in the nearby Medway River (pH 5.6). Blood concentrations of T and 11-KT have been directly correlated with gonadal development and sexual maturation in all salmonids investigated so far (Idler et al. 1971; Ozon 1972) rising to a peak at or near functional sexual maturity and declining thereafter. The 1981 findings strongly suggested a disturbance in sex hormone production and sexual maturation in Westfield fish. Our studies in subsequent years included the analyses of steroid hormones T, 11-KT, and 17α,20β-dihydroxy-4-pregnen-3-one (17,20-P) in the blood and corticosteroid hormone (e.g. cortisol) metabolism in vitro by head kidney interrenal tissue (adrenal homologue). Evidence of the significant role of T, 11-KT, and 17,20-P in the reproductive physiology of salmoniformes has increased since the isolation of these steroids in the early 1960's (Idler et al. 1960; Schmidt and Idler 1962). T and 11-KT are potent

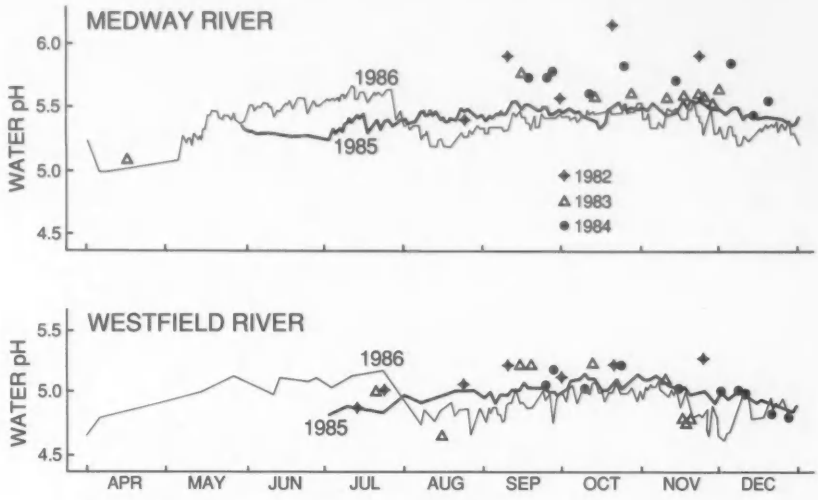

FIG. 1. Water pH of Westfield River and Medway River, Nova Scotia, 1982–86.

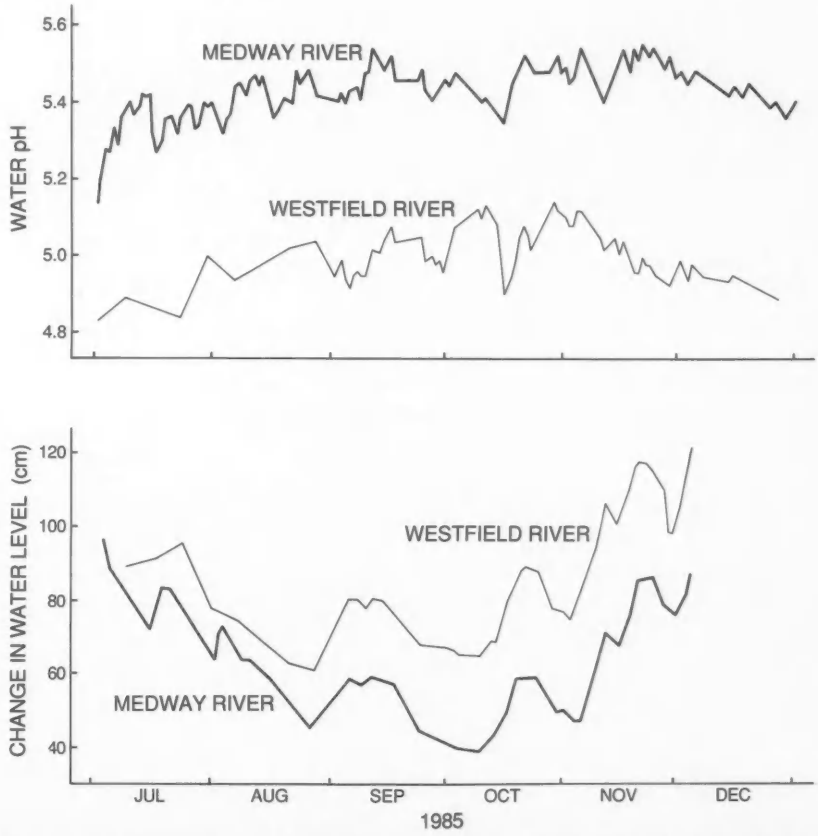

FIG. 2. Water pH and change in water level of Westfield River and Medway River during the Atlantic salmon holding period in 1985.

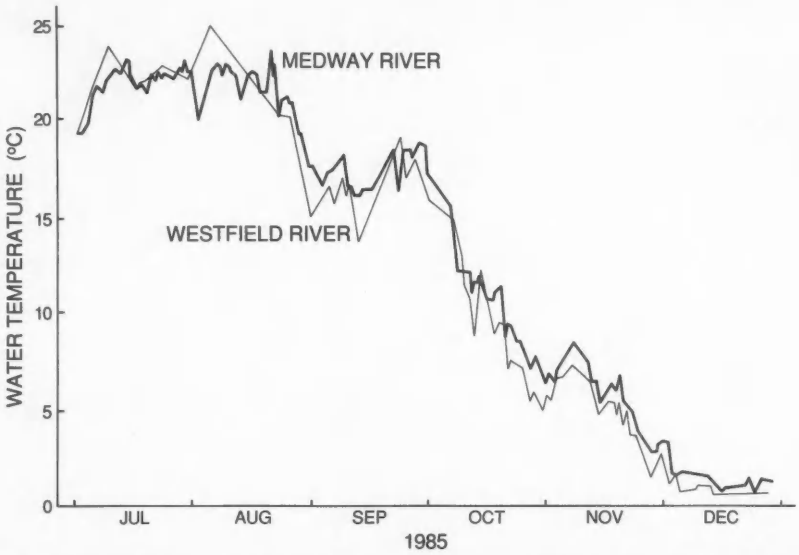

FIG. 3. Water temperature of Westfield River and Medway River during the Atlantic salmon holding period in 1985.

androgens for some teleosts and have a significant role in gonadal development and sexual maturation in salmonids (Ozon 1972). Recent evidence demonstrated the significance of 17,20-P as a follicular mediator of gonadotropin and its requirement in germinal vesicle breakdown (ovulation) and spermiation (milt formation) in salmonids as well as other fish (Jalabert et al. 1977, 1978; Campbell et al. 1980; Iwamatsu 1978; Nagahama et al. 1983; Ueda et al. 1983). Concentrations of blood corticosteroids, e.g. cortisol, and corticosteroid biosynthetic profiles change with physiological conditions such as stress, exercise, sexual maturity, osmoregulatory adaptation, proximity to death, or with experimental handling. Cortisol is the principal corticosteroid in the blood and in in vitro steroidogenesis by interrenal tissue of Atlantic salmon *Salmo salar* (Idler and Truscott 1972; Sangalang et al. 1972). We chose to study in vitro (head kidney interrenal tissue) rather than in vivo (blood) corticosteroid production to investigate stress hormone activity in the Atlantic salmon because blood concentrations can be extremely labile, fluctuating with handling alone whereas tissue biosynthetic profiles reflect changes in cellular structure and, within the limits of in vitro incubation techniques, provide a more accurate assessment of interrenal activity.

We conducted field studies from 1982 to 1984 in which we maintained salmon in floating cages in the Westfield and Medway Rivers through the last few months of their sexual maturation stages including spawning (Freeman et al. 1983; Freeman and Sangalang 1985) to determine reproduction, growth, and sex and stress hormone metabolism in these fish. In summary, the Westfield fish exhibited the following: (1) abnormal sex hormone and stress hormone metabolism, (2) lethargy, reduced appetite, and growth compared to fish held in the Medway River control, (3) depressed fecundity (number of eggs/g body weight) and elevated egg mortality compared to Medway fish, and (4) high mortality, occurring after spawning in the cold, acidic water. However, moribund fish recovered when transferred from the Westfield to the Medway River which was at the same temperature but had a higher pH. The above results gave evidence of the serious threat of the Westfield River environment on the reproductive capacity and, ultimately, survival of its salmon stock. Using the same techniques, we then investigated methods that could be used to mitigate the impacts of acidic precipitation in order to protect, or create refuge areas, for native salmon stocks at least until such time as abatement of this form of pollution occurs.

The effects of a high salt (sodium chloride)-containing diet were investigated in 1985. This approach was based on the observation (Beamish et al. 1975) that fish in acidic waters lose electrolytes, particularly sodium and chloride ions, via their gills and this loss of salts may be related to reproductive failures. We also studied the effects of liming Westfield river water in 1986. During 1982–1984, the Department of Fisheries and Oceans had already been looking into the technical feasibility of decreasing the acidity of some southern upland Nova Scotian rivers by application of limestone (Watt et al. 1984; White et al. 1984). Our objective was to study, in depth, the efficacy of limestone as a protective or ameliorative agent in reducing the impacts of acidification on sexually maturing salmon. Biologists at the Mersey Hatchery, Milton, Nova Scotia, have reported improved overall performance and survival of salmon maintained in acidic Mersey River water that had been treated with limestone.

This report describes the results of the 1985 and 1986 studies.

Study Sites and Experimental Conditions

Our area of study is underlain with granitic and hard, metamorphic rock and is, therefore, geologically sensitive to acid precipitation. Watt et al. (1983) have shown that more than 70% of the increase in acidity of the rivers in southwestern Nova Scotia was attributable to increased sulphate deposition. Riverine pH's generally reflect the pH of rainfall (3.5–6.0) and are the lowest in our study area. CANSAP (Canadian Network for Sampling and Precipitation, Department of the Environ-

ment, Canada) showed an annual mean pH of precipitation in the range 4.4–4.6. The pH profiles of the Medway and Westfield Rivers, 1982–1986 (Fig. 1) reflect the prevailing trends in precipitation, i.e. more acidic for the experimental site at Westfield and less so for the Medway site approximately 40 km southeast. Although the Medway River has a higher pH than the Westfield River, both rivers have the same hardness, ca. 4 mg $CaCO_3 \cdot L^{-1}$.

The pH of the Westfield River and Medway River from July to December, 1985, ranged 4.83–5.14 and 5.24–5.55, respectively. The pH of the Westfield River was higher in 1985 than in November, 1981 (pH 4.7). The pH of the Medway River was below 5.4 several times in 1985. Low riverine water pH was also coincident with higher water levels due to heavy rains (Fig. 2). Water temperatures of the two rivers were within 0.5–1°C of each other (Fig. 3).

Method

Fish

Two-year-old Atlantic salmon were purchased from a local fish farm (Ocean Farms Ltd., Upper Tantallon, Nova Scotia). These fish were River Phillip strain raised from eggs to the smolt stage at the Mersey Hatchery and then transferred to the fish farm where they were grown in seawater until purchased in late spring of the following year. The fish for the 1985 and 1986 experiments were from salmon-grilse cross-fertilizations.

For each year, 200 salmon, weighing 600–1200 g were selected, acclimated to freshwater in a nearby brook, then transferred by tank truck to a floating cage (2.4 m × 3.7 m × 1.8 m deep, 25 mm mesh netting) in the Medway River. After a minimum 1-wk holding period, the fish were anesthetized in tricaine methane sulfonate (MS 222):freshwater (1:20 000) and tagged with Carlin tags attached through the dorsal muscle just ventral to the anterior dorsal fin. One millilitre blood samples were obtained by caudal venipuncture into heparinized syringes. The weight and length of each fish were measured. Plasma was separated by centrifugation at 0–4°C. The blood plasma samples were analyzed by steroid radioimmunoassay to determine the sex of the fish (Sangalang et al. 1978) and basal levels of the steroids, T, 11-KT, and 17,20-P. Over the course of the holding period any diseased or dying fish were discarded.

1985

After holding in the Medway cage (as above) four equivalent groups, 23 males and 23 females of approximately the same body condition (body weight/body length), were distributed as follows: three in the Westfield River: Westfield (not fed), Westfield (Co-op diet), fed a commercial trout grower diet (6.2 mm, registration no. 810155, Atlantic Co-op Ltd., New Minas, Nova Scotia), Westfield (DFO high-salt diet), fed a high salt-containing diet (sodium chloride added to Atlantic salmon broodstock feed formula DFOH-85DB as modified by Dr. Santosh Lall, Halifax Fisheries Research Laboratory, Department of Fisheries and Oceans), and one in the Medway River: Medway-(Control), fed the commercial trout grower Co-op diet. The DFO diet had ca. the same proportions of vitamin premix (1.0%), mineral premix (6.5%), minimum crude fat (9%) and minimum crude protein (40.8% supplied primarily from herring and soybean meal) as the Co-op diet. The vitamin premix in the DFO diet had 9,000 $IU \cdot kg^{-1}$ Vitamin A (as acetate) and 5,000 $IU \cdot kg^{-1}$ Vitamin D_3 while the Co-op diet had certified minima of 8,000 $IU \cdot kg^{-1}$ and 3,000 $IU \cdot kg^{-1}$, respectively. The DFO diet contained 3.0% sodium chloride compared to 0.3% for the Co-op diet. Both diets contained 3.0% calcium. All fish, with the exception of the Westfield (not fed) group, were fed once daily until the middle of October and once every other day thereafter.

1986

Salmon were divided into three groups of ca. 40 fish each of equal numbers of each sex. One group was retained in the Medway and two groups transferred to the Westfield where one (Westfield-untreated) was placed in a cylindrical 3600 L fibreglass tank lined with the same netting as used in the cages receiving flowing, untreated river water and the other (Westfield-limed) in a similar tank receiving river water that had been passed through 160 kg of ca. 2 cm diameter marble chips (limestone). Timber barrages were constructed within the Westfield River of such a design as to be able to maintain a headwater pond and regulate the flow of the river water into the fish holding tanks. River water flowed into the tanks at 23–30 $L \cdot min^{-1}$ through wooden troughs 15 cm deep × 30 cm wide × 30–33 m long. Water flow was regulated by opening or closing gateways at the two barrages constructed 20 m and 30 m upstream of the tanks and/or by opening or closing cutouts on the sides of the troughs. The limestone beds were cleaned and raked daily; water pH in the limed tank was measured before and after raking. Dissolved oxygen and ammonia were not determined, but no visual evidence of stress or increased mortality were noted during warmer, low water periods which occurred early in the experimental period.

In each year of the study, the water pH, temperature, and height were measured daily, whenever possible. At the beginning of the holding experiments and at 2–3 wk intervals thereafter, blood samples and weight and length measurements were obtained from each fish after light anesthetization in MS222:freshwater (1:30 000). The females were checked for ovulation twice a week during the spawning season. At spawning time, eggs from females in a group were pooled and fertilized with mixed sperm from ripe males in the same group. Mean egg diameter for each fish was measured. Fecundity (number of eggs per g body weight) for each group was determined. The eggs were brought to the Mersey Hatchery where they were incubated, manually counted, and tended daily to the hatch stage. The holding experiments were terminated at post-spawning around the beginning of the second week of December at which time investigators from other research institutes participated. Blood and other tissue samples were obtained in the field. Usually, 12 fish from each group, six males and six females, were killed by exsanguination and a quick blow to the head after anesthetization in phosphate-buffered MS222: freshwater (pH 7; 1:20 000). For our steroid metabolic studies, head kidney and gonadal tissues were quickly excised, a small 1-cm piece of mid-section fixed in 10% phosphate-buffered formalin, and the remainder immersed in liquid N_2, then stored over dry ice for transportation back to the laboratory where they were stored at −40°C until used for in vitro steroidogenesis studies.

Plasma Steroid Analysis

Plasma concentrations of T, 11-KT, and 17,20-P were determined by radioimmunoassay according to the methods of Sangalang and Freeman (1977; 1987).

TABLE 1. Weight change in Atlantic salmon held in the Westfield and Medway Rivers from August to before spawning in November 1985.

River and group	Diet	Initial weight g, mean ± SD range	Weight change g, mean ± SD range	Percent mean loss
Medway	Co-op	775 ± 125 575–1075	25 ± 25 −75 to +75	3.2
Westfield	Not fed	770 ± 75 550–750	100 ± 25 −170 to −65	14.3
	Co-op	750 ± 100 550–850	100 ± 50 −175 to 0	13.3
	DFO high-salt	850 ± 225 575–1250	75 ± 75 −175 to −85	8.8

In Vitro Steroidogenesis

Head kidneys and gonads were freed of extraneous tissues and pooled according to group, sex, and maturation stage as observed from gross examination of the gonads. The tissues were minced and mixed to form a homogeneous mixture. One-g samples were incubated with equimolar amounts (0.035 μmole) of radioactive precursors, [^{3}H]-pregnenolone (370 KBq) and [^{14}C]-progesterone (74 KBq) (New England Nuclear Canada) in the presence of an NADPH-generating system for 4 h at 8°C. The initial precursor ^{3}H:^{14}C ratio was approximately 5:1. The methods have been described elsewhere (Sangalang et al. 1972).

Steroid metabolites were extracted from the tissue incubations with dichloromethane. One-tenth of each crude extract was chromatographed on thin-layer chromatoplates coated with 0.5 mm Silica Gel $HF_{254+226}$ (Merck Darmstadt, Germany) first in solvent system hexane:ethyl acetate (4:1, v/v) and then in chloroform:methanol:water (94:6.0:0.5, v/v/v). For an imprint of the radioactive steroid metabolic profiles, the thin-layer chromatoplates were exposed to a no-screen medical X-ray film for usually no less than 48 h in a light-proof box. The films were developed according to the manufacturer's specifications. Details of methods have been described by Freeman and Sangalang (1977). Steroid metabolites were identified by sequential thin-layer and paper chromatography, acetate derivative formation and recrystallizations to constant ^{3}H:^{14}C isotope ratios (Axelrod et al. 1965).

Radioactivity was measured with a Packard liquid scintillation spectrometer, Model 300 (Packard Instruments Ltd., Chicago) equipped with automatic external standardization.

Results and Discussion

All groups of salmon showed weight losses from August to early November 1985 when some fish were ready for spawning. The Westfield fish lost, on average, 8.8–14.3% body weight while the Medway fish lost only 3.2% (Table 1). This was the first time we observed weight loss in the Medway fish. The Westfield (not fed) group lost the most weight (14.3%).

Only three fish were removed from the Medway cage while 10, 8, and 9 were removed from Westfield (not fed), (Co-op diet), and (DFO high-salt diet), respectively. The higher losses, general lethargy, and lack of appetite of fish held in the Westfield River water reflected the stressful and adverse conditions of their acidic environment. Although we did not measure swimming activities, we had observed that the fish fed the DFO diet were more active and responsive than the fish in the other two Westfield groups.

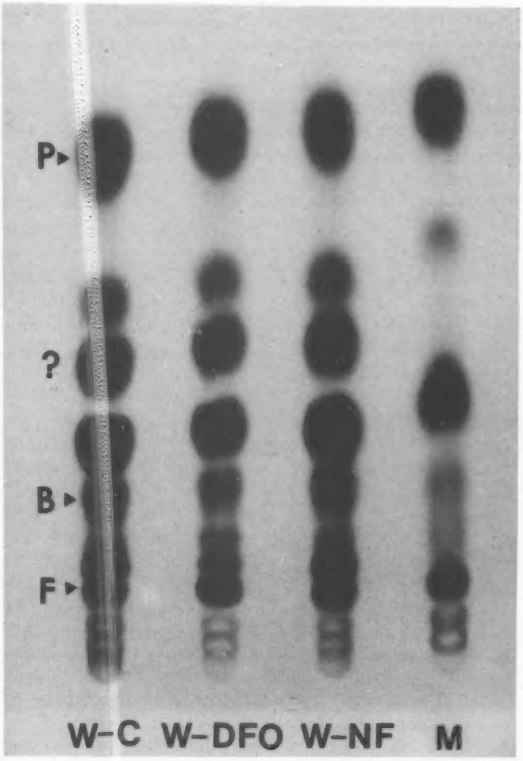

FIG. 4. Autoradiogram of the thin-layer chromatogram of extracts of radioactive steroid metabolites from Atlantic salmon head kidneys (males only) incubated with [^{3}H]-pregnenolone plus [^{14}C]-progesterone, in vitro. M, Medway group fed Co-op diet; W-NF, Westfield group, not fed; W-C, Westfield group, fed Co-op diet; W-DFO, Westfield group, fed DFO high-salt diet. The dark spots indicate positions of radioactive steroid metabolites. Note the high intensity of spots isopolar with cortisol (F) and corticosterone (B) and an unknown metabolite (?) in all the Westfield groups compared to the Medway group. P indicates position isopolar with precursors, pregnenolone and progesterone.

The mean egg diameter of the Medway female salmon were significantly (t-test, $P<0.001$) larger than all Westfield group values (Table 2). The significant differences in egg sizes between the Medway and Westfield fish may reflect physiological differences such as electrolyte balance or biochemical composition. Fecundity was also significantly (t-test, $P<0.001$) higher in the Medway fish than in the Westfield (not fed) and Westfield (Co-op diet), but not in the Westfield (DFO high-salt diet) group. There was a higher ratio of spawning females to immature females in the Medway and Westfield (DFO high-salt diet) groups compared to the other two Westfield groups. As in previous years, there was a high mortality of post-spawned Westfield fish when left in the cold (<5°C) acidic water and moribund fish recovered when transferred to the Medway facility. It is possible that this observation may be related to the reported scarcity of repeat spawners in the Westfield River (Watt et al. 1983).

TABLE 2. Spawning data for female Atlantic salmon held in cages in the Medway and Westfield Rivers from mid-August to early November, 1985.

River and group	Diet	Mature/ Surviving females	Weight, g surviving females Mean ± SD	Total egg production per group	Egg diameter mm, Mean ± SD	Fecundity (number eggs/ g body weight) Mean ± SD
Medway	Co-op	17/20	700 ± 130	16,093	5.32 ± 0.04	1.38 ± 0.10
Westfield	Not fed	7/13	625 ± 75	3,778	4.37 ± 0.03	0.85 ± 0.05
	Co-op	9/15	650 ± 100	7,726	4.70 ± 0.04	1.24 ± 0.08
	DFO high-salt	11/14	800 ± 200	12,433	4.60 ± 0.05	1.34 ± 0.08

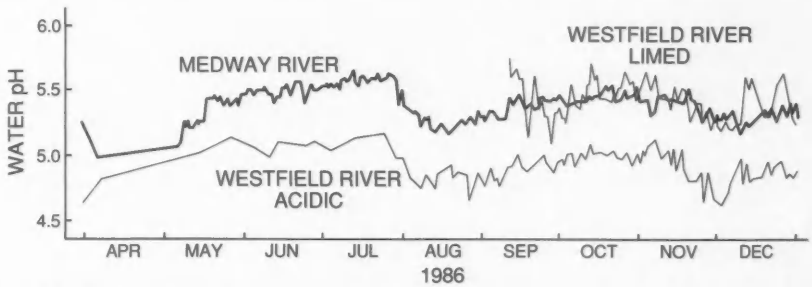

FIG. 5. The effects of liming on the water pH of Westfield River water at the Atlantic salmon holding site. Liming elevated the pH of Westfield River water to values similar to or slightly higher than those of the Medway River.

The fecundity of the females may be related to their blood peak levels of 17,20-P, the hormone necessary for final oocyte maturation and spermiation in salmonids. Blood levels of 17,20-P covered a wide range within each group with the highest peak levels occurring in the Medway and Westfield (DFO high-salt diet) groups on November 25, and 7, respectively. The mean peak levels ($ng \cdot mL^{-1}$ plasma ± SD) of 17,20-P in the females were 95.2 ± 22.4, 53.9 ± 16.0, 60.7 ± 21.0, and 71 ± 21.0 for the Medway, Westfield (not fed), Westfield (Co-op diet), and Westfield (DFO high-salt diet) groups, respectively. Thus, the results indicated a relationship between fecundity in the females and sex hormone levels. Blood levels of 17,20-P in the Medway group were significantly higher (F-test, $P<0.001$) than the Westfield (not fed) and (Co-op diet) groups, but not the Westfield (DFO high-salt diet) group. There was a ca. 2-wk delay in the peaking of this hormone in two-thirds of the Westfield (not fed) and (Co-op diet) fish although T and 11-KT peaked at or just before spawning in all groups as is normal for salmonids. Since peaking of the blood level of 17,20-P is necessarily coincident with germinal vesicle breakdown (ovulation) in the female and the period of spermiation in the male, the delay in peaking of 17,20-P levels in the Westfield — (not fed) females could inhibit their spawning success or reproductive performance. It is apparent that the DFO high-salt diet prevented an adverse effect on sex hormone metabolism in the Westfield fish.

Egg mortalities were high in all groups: 79.1%, 98.9%, 100%, and 84.8% for the Medway, Westfield (not fed), (Co-op diet), and (DFO high-salt diet) groups, respectively. This was thought to be due to handling stress from being bled just before spawning and/or to the cold air temperature (<0°C) at the time of the spawnings and cross-fertilizations.

The above results show that diet can play an important role in the management of fish in acidic waters. The general performance of the Westfield fish on the high salt-containing diet was maintained at almost the level observed in the Medway fish but it is not known whether this was due solely to a compensatory effect on blood electrolyte imbalance or to other effects.

Corticosteroid metabolic patterns, in vitro, in the head kidneys of the Westfield fish showed an increased production of cortisol (Fig. 4) as had been observed in previous years (Freeman and Sangalang 1985). A consistent appearance of the abnormal 17-deoxycorticosteroid metabolite, corticosterone, gives support to our speculation that this steroid may provide the mechanism to regulate cortisol biosynthesis (Idler and Truscott 1972) as well as control osmoregulation in response to stress. The in vitro biosynthesis of corticosterone, coupled with a high production of the stress hormone, cortisol, by the head kidneys reflected a true disturbance in the normal hormonal milieu of the Atlantic salmon.

Steroidogenesis in the testes and ovaries did not show any significant differences between groups except for a somewhat lower, but not significant, reduction in the yield of 17,20-P in the Westfield (not fed) group. Analysis of the metabolites confirmed the identity of 17,20-P as the principal metabolite in both testes and ovaries but the yields were low (<0.1%), probably due to the onset of gonadal regression in these tissues at the time of sampling. The ^{3}H:^{14}C ratios did indicate a difference in the biosynthetic pathway to 17,20-P between testes and ovaries.

1986

Liming increased the pH of Westfield water to values (pH 5.1–5.9) similar to or slightly higher than those of the Medway River (Fig. 5). The pH of the untreated Westfield water ranged 4.7–5.2. As in previous years, water pH generally fell in both

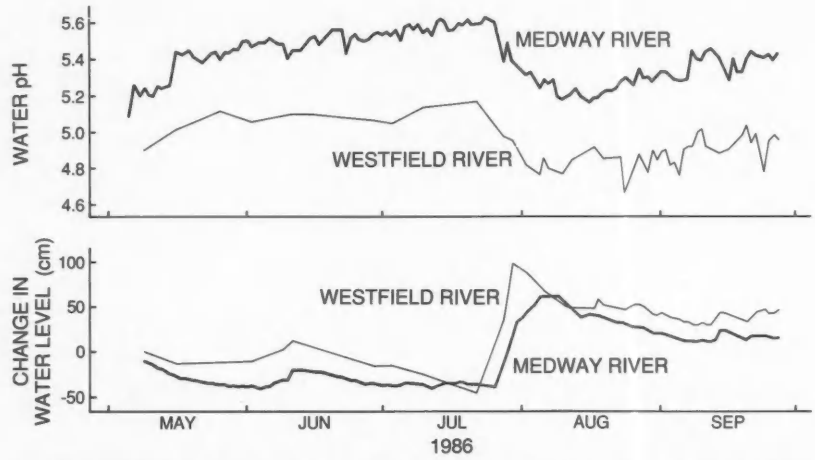

FIG. 6. Water pH and change in water level of the Westfield River and Medway River during the Atlantic salmon holding period in 1986.

TABLE 3. The effects of limestone treatment of Westfield River water on sexual maturation in female Atlantic salmon, 1986.

River and group	Number spawned/ total number of females	Egg diameter mm; mean ± SD	Number of eggs	Fecundity (number of eggs/ g body wt. ± SD)
Medway	17/20	5.03 ± 0.04	31,500	1.77 ± 0.08
Westfield				
Untreated	11/20	4.73 ± 0.02	15,790	1.55 ± 0.05
Limed	13/17	4.91 ± 0.02	23,200	1.72 ± 0.08

rivers and was coincident with high water levels (Fig. 6), particularly those resulting from heavy rains from the southwest.

The fish in the limed water lost less weight (44.0 g or 4.6%), on average, than the fish in the untreated water which lost an average of 112 g or 11.7% from August to late October, the time of first spawning. The Medway fish gained 48 g or 5.2%, on average, which is not significantly different from the weight change in the limed Westfield fish. The fecundity (Table 3) of the Westfield females and the number of females that spawned increased in the limed water. The egg mortalities for Medway, Westfield-untreated, and Westfield-limed groups from the time of spawning until the end of January, 1987 were 18.3%, 60.3%, and 34.2%, respectively, indicating a significant and favourable effect of the limestone treatment on reproduction.

Compared to 1985, the blood peak levels of 17,20-P in the Medway and Westfield fish varied widely giving no significant differences between groups (Table 4). Peak levels in 1985 were significantly lower (F-test, $P<0.001$) in the Westfield than in the Medway fish. However, in 1986, although the blood levels of 17,20-P (14.67–172.0 ng·mL^{-1}), T (26.46–125.1 ng·mL^{-1}) and 11-KT (23.06–122.7 ng·mL^{-1}, males only) did not differ significantly between groups, the times of peak blood concentrations did. Limestone treatment stimulated early peaking of T: 100% of the males gave peak levels on October 21 compared to 78% and 75% of the Medway and Westfield-untreated. However, limestone treatment delayed peaking in some males and stimulated peaking in some females (Table 4). At spawning time, during milt formation, blood levels of 17,20-P gradually rose to a maximum level in males and then declined gradually. By contrast, blood levels of 17,20-P in females rose dramatically at ovulation then fell drastically afterward. The spawning time for the majority of fish in all groups was on or before November 25 but none of the fish in the untreated Westfield water gave peak levels of 17,20-P or spawned before this date. There were no females that spawned

TABLE 4. Effects of limestone treatment of Westfield River water on blood sex hormone levels in Atlantic salmon, 1986.

River and group	Peak blood levels range (ng·mL^{-1} plasma)	Sampling day: ratio of number of fish with peak levels to total number			
		Oct 21	Nov 7	Nov 25	Dec 5
Testosterone					
Medway					
Male	44.65–83.91	15/19	4/19	0	0
Female	31.94–92.38	0	12/20	0	0
Westfield					
Untreated					
Male	45.12–96.58	15/20	5/20	0	0
Female	26.46–125.1	0	12/21	9/21	0
Limed					
Male	48.30–85.32	20/20	0	0	0
Female	36.10–83.43	1/19	14/19	4/19	0
11-Ketotestosterone (males only)					
Medway	25.67–122.7	2/16	0	0	
Westfield					
Untreated	25.63–165.2	7/18	11/18	0	0
Limed	23.06–115.1	12/17	4/17	1/17	0
17α,20β-dihydroxy-4-pregnen-e-one					
Medway					
Male	41.90–127.5	0	2/19	13/19	5/19
Female	14.98–161.9	0	6/20	14/20	0
Westfield					
Untreated					
Male	20.84–131.1	0	0	16/20	4/20
Female	19.39–125.7	0	0	19/21	2/21
Limed					
Male	14.67–172.0	0	0	6/20	14/20
Female	15.04–164.1	0	3/19	14/19	2/19

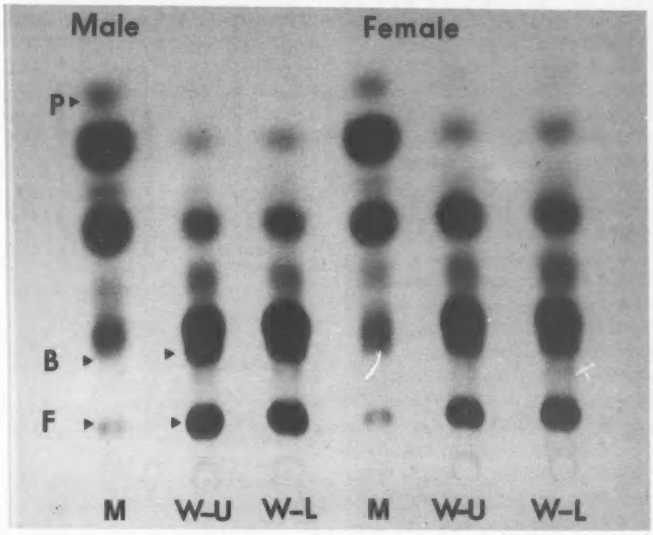

FIG. 7. Autoradiogram of the thin-layer chromatogram of extracts of radioactive metabolites from Atlantic salmon head kidneys incubated with [^{3}H]-pregnenolone plus [^{14}C] progesterone, in vitro. M, Medway; W-U, Westfield-untreated; W-L, Westfield-limed. The steroid metabolites, cortisol (F) and corticosterone (B) and precursor, progesterone (P) are shown isopolar with dark spots marking radioactive areas. Note the more intense radioactivity in the cortisol (F) fractions of the head kidney extracts from all Westfield fish compared to those of the Medway fish.

TABLE 5. Isotope ratios (^{3}H:^{14}C) of steroid metabolites from [^{3}H]-pregnenolone plus [^{14}C]-progesterone incubated with head kidneys (interrenals) of Atlantic salmon, in vitro, 1986.

	Cortisol	Corticosterone	17,20-P	Progesterone
Male				
Medway	0.575	<0.01	0.986	0.055
	0.505	<0.01	0.929	0.058
	0.586	<0.01	0.959	0.064
Westfield				
Untreated	1.40	<0.01	2.38	0.087
	1.31	<0.01	2.02	0.096
	1.31	<0.01	1.95	0.094
Limed	1.21	<0.01	2.23	0.111
	1.13	<0.01	1.92	0.101
	1.12	<0.01	1.98	0.110
Female				
Medway	1.82	<0.01	4.05	0.059
	1.88	<0.01	3.78	0.063
	1.85	<0.01	3.72	0.069
Westfield				
Untreated	1.05	<0.01	1.71	0.109
	1.04	<0.01	1.71	0.098
	1.05	<0.01	1.53	0.109
Limed	0.797	<0.01	1.16	0.093
	0.771	<0.01	0.923	0.100
	0.777	<0.01	0.956	0.111

after November 25 in the Medway group but there were two females that spawned on December 5 in each Westfield group, suggesting a trend toward delayed ovulation in the Westfield females. It is obvious that for successful spawning the period of ovulation (i.e. peak blood levels of 17,20-P) in the females must occur during milt formation (rise in the production of 17,20-P) in the males. In early December, 1986, the river water temperatures were <5°C. The somewhat prolonged production of 17,20-P in the males and early peaking of this hormone in the females in the limestone-treated Westfield water at a time when the water temperatures were >5°C may explain the successful cross-fertilization programs at the Mersey Hatchery where limestone is used to treat the acidic river water.

There were no significant differences in the yields of in vitro steroid metabolites isopolar with T, 11-KT, and 17,20-P in the ovaries and testes of the fish in all groups. The yields of these steroid metabolites were very low (<0.1%), presumably due to the post-spawned state of the gonads. The identities of these steroid metabolites could not be confirmed due to insufficient radioactivity.

The marked and significantly higher production of cortisol and corticosterone in the head kidneys of all Westfield fish compared to Medway fish (Fig. 7) confirmed the previous year's findings that the Westfield fish were under severe stress. The Westfield fish were highly stressed compared to Medway fish whether held in floating net cages or in tanks. Such stress had not been noted in later experiments in which salmon were held in a tank in the Medway similar to the holding tanks in the Westfield (pers. obs.). The ^{3}H:^{14}C ratios (Table 5) of the steroid metabolites again showed a difference in the biosynthetic pathways to cortisol and 17,20-P between Westfield and Medway fish. The lower isotope ratios of cortisol in the head kidneys of male Medway fish indicated a lower conversion of the ^{3}H precursor, pregnenolone, or a higher conversion of the ^{14}C precursor, progesterone, compared to the Westfield groups. By contrast, the isotope ratios of cortisol in the head kidneys of female fish were higher in the Medway group than in the two Westfield groups. The isotope ratios of steroid metabolites in the head kidneys of all groups of fish, however, were lower than the initial steroid precursor ratio of 5:1 which also suggests the predominance of progesterone as the precursor and the delta-

4-3-keto pathway as the favoured route of biosynthesis. The biosynthesis of corticosterone was mainly from progesterone in all groups as indicated by the very low $^{3}H:^{14}C$ ratios (<0.001) in the Westfield groups than in the Medway group. The yields of corticosterone were >3% in the Westfield groups and <0.1% in the Medway group. It would be interesting to know the correlation between head kidney morphology and in vitro corticosteroid metabolism. Thus, although limestone treatment maintained the reproductive performance somewhat and stimulated early peaking of sex hormones, it was not able to prevent the stress hormone imbalance in the Westfield fish.

In summary, a sodium chloride enriched diet or limestone treatment of the acidic river water may be worthwhile for mitigation, but only in the short term since neither fully prevented all adverse effects observed when salmon were held in untreated, acidic Westfield River water. The Westfield fish were still severely stressed, in spite of diet or water treatment. This is particulary intriguing, given that the limestone treatment restored the pH of the Westfield River water to that of the Medway River. This suggests that either other toxic materials are present in untreated Westfield River water in addition to hydronium ion, or that limestone treatment itself has an adverse effect.

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Responses of Plasma Electrolytes, Thyroid Hormones, and Gill Histology in Atlantic Salmon (*Salmo salar*) to Acid and Limed River Waters

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Brown, S. B., R. E. Evans. H. S. Majewski, G. B. Sangalang, and J. F. Klaverkamp. 1990. Responses of plasma electrolytes, thyroid hormones, and gill histology in Atlantic salmon (*Salmo salar*) to acid and limed river waters. Can. J. Fish. Aquat. Sci. 47: 2431–2440.

Sexually maturing Atlantic salmon, *Salmo salar*, were held, in the acidic (pH range 4.7–5.2) Westfield River, Nova Scotia and in the nearby, less acidic (pH range 5.2–5.6) Medway River. Exposure to Westfield River water in 1985 (149 d) and 1986 (126 d) reduced plasma osmolality, Na^+, Cl^-, and Ca^{++} (in females only) concentrations of post-spawning fish compared to those in fish held in the Medway River. There were coincidental increases in plasma K^+, glucose, and unidentified osmotic fraction (UOF). Gill tissue showed hyperplasia of primary lamellae epithelium. Together, these findings indicate compromised ionoregulatory ability. Decreased plasma T_3 (3,5,3′-triiodo-L-thyronine) suggests altered thyroid function. Westfield River water did not affect plasma T_4(L-thyroxine) or protein concentrations. An unintentional handling stress caused even more severely depressed plasma ions and more elevated plasma glucose in Westfield fish in 1985 relative to 1986; Medway fish largely recovered from this stress. These observations indicate that acid-exposed fish may be more sensitive to additional stressors. Limestone treatment of Westfield River water (elevating its pH to Medway values) ameliorated ionoregulatory ability but did not affect plasma T_3 and Ca^{++} (female). A high salt diet (3% NaCl) failed to protect salmon from the effects of acidic water.

Des saumons de l'Atlantique, *Salmo salar*, en voie de maturation sexuelle, ont été placés dans l'eau acide (pH 4,7–5,2) de la rivière Westfield (Nouvelle-Écosse) et dans la rivière Medway avoisinante, moins acide (pH 5, 2–5,6). Une exposition à l'eau de la rivière Westfield en 1985 (149 j) et en 1986 (126 j) a réduit l'osmolalité du plasma, les concentrations (chez les femelles seulement) de Na^+, Cl^- et Ca^{++}, après la fraye, comparativement à celles des poissons placés dans la rivière Medway. Par coïncidence, on a enregistré des augmentations du K^+, du glucose et de la fraction osmotique non identifiée du plasma. Le tissu branchial présentait une hyperplasie de l'épithélium des lamelles primaires. Tous ces résultats révèlent une aptitude ionorégulatrice altérée. Une diminution de la T_3 (3,5,3′-triiodo-L-thyronine) laisse supposer une altération de la fonction thyroïdienne. L'eau de la rivière Westfield n'a pas affectée la concentration de la T_4 (L-thyroxine) ni les teneurs en protéines dans le plasma. Une mauvaise manipulation involontaire a causé une baisse encore beaucoup plus importante des ions plasmatiques et élévation plus forte du glucose plasmatique chez les poissons de la rivière Westfield en 1985 par rapport à 1986; les poissons de la rivière Westfield se sont sensiblement bien rétablis de cette agression. D'après ces observations, les poissons en milieu acide peuvent être plus sensibles à d'autres agents de stress. Le traitement à la chaux de la rivière Westfield (en élevant son pH à celui de la rivière Medway) a augmenté sa capacité ionorégulatrice mais n'a pas modifié la T_3 et le Ca^{++} du plasma (femelles). Un régime alimentaire riche en sel (3% NaCl) n'a pas protégé les saumons des effets de l'eau acide.

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Acidification of poorly buffered freshwater systems threatens fisheries resources in eastern North America (Haines 1981). In particular, the upland region of southern Nova Scotia has received extensive mineral acid deposition from atmospheric emissions originating in the northeastern United States (Watt et al. 1983). Almost 50% of the production capacity for Atlantic salmon (*Salmo salar*) in this area has been lost due to acidification (Watt 1987).

Causes for declines in salmonid populations due to acidification are complex. Factors may include disturbance of the ecosystem resulting in partial or total disappearance of food sources (Mills et al. 1987) and significant mortalities of eggs and fry (Nelson 1982; Peterson et al. 1982; Lacroix 1985*a*; Peterson and Martin–Robichaud 1986). However, acidification also has direct effects on the physiology of fish; the most important physiological disruptions involve ionoregulation and associated growth and reproduction (Leivestad 1982; Wood and McDonald 1982; Freeman and Sangalang 1985; Lacroix et al. 1985; Tam and Payson 1986; Tam et al. 1987).

The purpose of this study was to examine sensitive acid stress indicators, determined in previous laboratory studies (Brown et al. 1984; Giles et al. 1984; Evans et al. 1988), in fish exposed to acidic conditions in natural systems. To achieve these conditions we evaluated the physiological and histological consequences of holding sexually maturing Atlantic salmon in acidic

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water (pH range 4.7–5.2) of the Westfield River and the less acidic water of the Medway River (pH range 5.2–5.6). We quantified depressive effects of Westfield River water on blood ions and the active thyroid hormone (3,5,3′-triiodo-L-thyronine) in addition to acid-induced gill hyperplasia and elevated plasma glucose in post-spawned fish. The mitigative effects of liming Westfield River water or feeding a high-salt diet were also examined.

Materials and Methods

Experimental Sites

Holding cages and tanks were constructed in the Medway River and a tributary, the Westfield River (Sangalang et al. 1990). The sites were located in the upland region of southwestern Nova Scotia (Fig. 1). The pH of each river was recorded daily. Water was collected in mid-December and total aluminum, cadmium, calcium, copper, iron, magnesium, manganese, zinc, and dissolved organic carbon determined by standard methods at the Freshwater Institute Chemistry Laboratory.

The pH (range 4.7–5.2) of the Westfield River was 0.3 to 0.6 lower than the pH (range 5.2–5.6) of the Medway River (Fig. 2). In 1986, liming restored pH of Westfield River water to values observed in the Medway River. Calcium levels averaged 25 μmol/L in the Medway River and 17 μmol/L in the Westfield River. Liming Westfield River water increased calcium levels to 32 μmol/L. Other measured constituents were not altered by lime treatment and were similar to those described previously for the rivers (Watt et al. 1983; Lacroix 1985a,b; Lacroix and Townsend 1987).

Experimental Fish

Sexually maturing 2-yr-old Atlantic salmon (River Phillip stock), weighing 600–1200 g, were purchased from a local fish farm (Ocean Farms Ltd., Upper Tantallon, N.S.) in 1985 and 1986. After acclimation to freshwater, the fish were initially transported to the Medway River and placed in floating fish cages (3.7 m × 2.2 m × 1.8 m deep, 25 mm mesh netting) (Freeman and Sangalang 1985).

Post-spawned fish mortality in the more acid Westfield River was 30%. There were no mortalities of post-spawned fish held in the Medway River or in limestone treated Westfield River water.

Experimental Protocol

1985

On July 15, after a minimum acclimation of 1 wk, the salmon were separated into four groups (Medway, Westfield, West-salt fed, and West-unfed) of 46 fish having equal numbers of each sex. The Medway group remained caged in the Medway River and was fed a commercial trout grower (Co-op diet, 0.3% NaCl). The other three groups were placed into cages in the Westfield River. The Westfield River groups were treated as follows: the Westfield group was fed the Co-op diet; group West-salt was fed a high salt diet (3.5% NaCl); the West-unfed group was not fed. All other details on dietary constituents are given in Sangalang et al. (1990). The fish were fed once daily until mid-October and once every other day thereafter.

1986

On August 5, salmon were divided into three groups of 40 fish designated Medway, Westfield, and West-limed. The

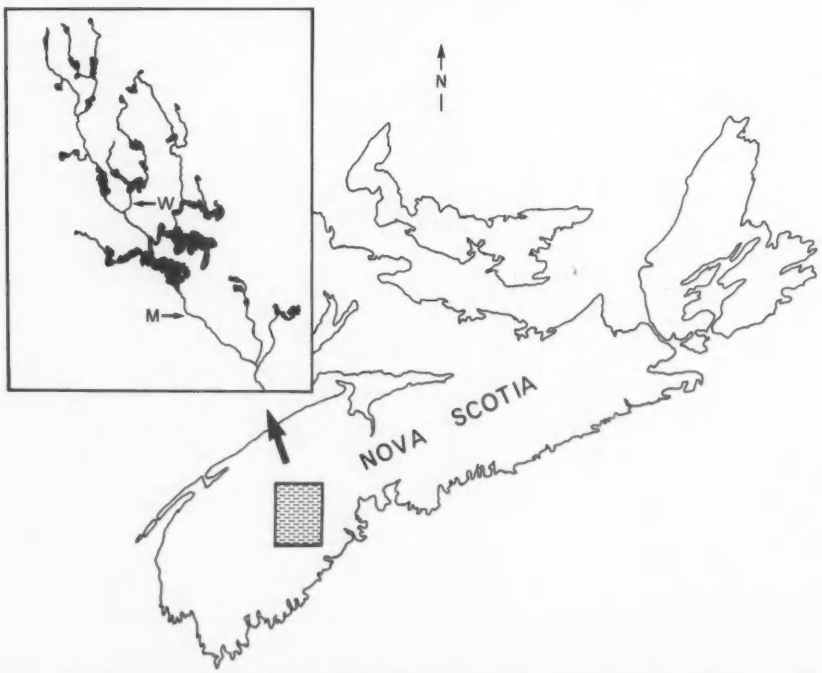

FIG. 1. Nova Scotia rivers where maturing Atlantic salmon were held between July and December 1985 and 1986. The Medway River and Westfield River are indicated by M and W, respectively.

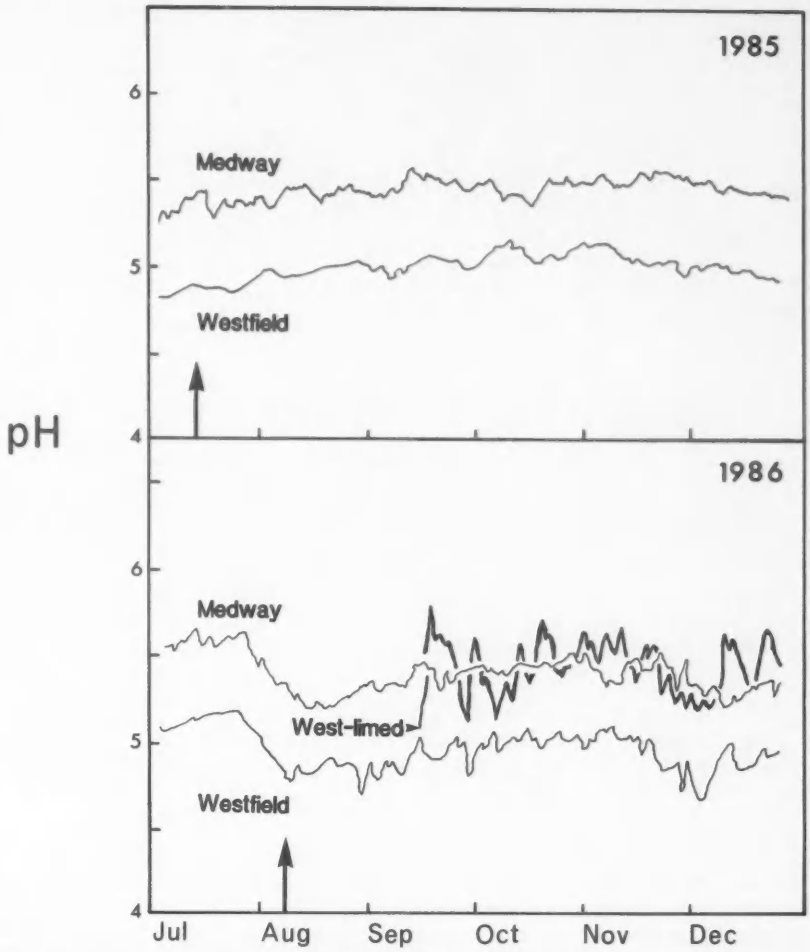

FIG. 2. Water pH of Medway River and Westfield River in 1985 and 1986. The times when fish were placed into the rivers are indicated by vertical arrows.

Medway group was held in a floating cage in the Medway River. The other groups were moved to tanks located in a branch of the Westfield River (Sangalang et al. 1990). Each tank received river water at 23–30 L/min. Starting September 10, the West-limed group received river water that was passed through and over crushed limestone which was raked daily. The Westfield group received untreated Westfield River water. All fish were fed the Co-op diet daily.

1987

A separate experiment was conducted in the Medway River to determine if differences existed between fish held in floating cages and tanks receiving river water. A tank site similar to that used in the Westfield River was constructed on the Medway River. On July 31, salmon were transported to the Medway River. Starting October 1, fish were separated into two groups of 40 fish having equal number of each sex. One group was held in a floating cage while the other was held in a submerged tank similar to those at the Westfield River site in 1986. Both groups of fish were handled in a similar fashion and fed the Co-op diet.

Total exposure times of Atlantic salmon to Westfield River water were 149 d and 126 d in 1985 and 1986, respectively. In 1986, the West-limed group was exposed to Westfield River water for 35 d before receiving limestone-treated water for the last 91 d of the experiment. In 1987, the Medway tank and Medway cage groups were held for 73 d.

Blood sampling

Before the terminal sample described below, the fish were lightly anesthetized with 35 mg/L tricaine methanesulphonate (TMS), weighed, measured, and 1 mL of blood removed at the beginning of the experiment and 0.5 mL at 2- to 3-wk intervals (Sangalang et al. 1990).

Unfortunately, in 1985 the fish were handled and bled only 1 or 2 d before our terminal sampling. In the following year, a 2- to 3-wk recovery period was allowed before terminal sampling.

After the final bleeding for a reproductive hormone study (Sangalang et al. 1990) in early December each year, 12 post-spawned salmon from each group (6 males, 6 females) were

sacrificed by immersion in pH-neutralized TMS (200 mg/L). Within a few minutes of being immobilized, the fish were bled from the caudal vessels using ammonium-heparinized 10-mL syringes and with 18-gauge needles. Plasma was immediately separated by centrifugation and frozen on dry ice in sterile 10-mL polypropylene tubes. Upon return to the laboratory plasma samples were stored at −120°C until analyzed.

Plasma measurements

Measurements of thyroxine (T_4), 3,5,3′-triiodo-L-thyronine (T_3), glucose, protein osmolality, Na^+, K^+, and Cl^- concentrations in plasma were performed according to previously described methods (Brown et al. 1984; Brown et al. 1986). Plasma Ca^{++} concentrations were estimated using a Sigma® test kit (No. 586) as described by Scherer et al. (1986).

Histological techniques

After fish were bled, the second gill arch (right holobranch) was removed from each fish. Each arch was fixed by immersion in Bouin's fixative and embedded in wax (Evans et al. 1988). Randomly selected sections of the central areas of the primary lamellae from each fish were examined. As indicators of epithelial hypertrophy or atrophy, measurements were made on three groups of 10 bilateral epithelial widths (5 on each side of the filament axis) on each fish (Evans et al. 1988).

Statistical analysis and calculations

Bartlett's test for homogeneity of variance was applied to the data and, where required, data were transformed according to Taylor's power law (Southwood 1978) to obtain more uniform variances. For clarity of presentation, untransformed arithmetic means and standard errors are given in the Results section. Analyses of variance were computed using the GLM procedure of the Statistical Analysis System (SAS Institute Inc. 1982). A three-way factorial arrangement using year, group, and sex was applied to test for significant differences in fish from the terminal sampling in December. Differences between group means were examined using Tukey's Studentized Range test. A probability level of < 0.05 was considered significant. Temporal data for blood ions in 1986 represent measurements on plasma samples from 10 representative salmon (5 males, 5 females) taken at each sampling interval of the reproductive hormone study (Sangalang et al. 1990). For individuals that were sampled repeatedly, the analytical approach of Rowell and Walters (1976), where contrasts are analyzed through a time series, was used to determine significant responses.

Condition factor (K) was calculated for 1985 and 1986 data using $k = 100\ w/l^3$ (Bagenal and Tesch 1978), where w is weight (g) and l is fork length (mm).

Assuming complete dissociation of Na^+ and Cl^-, the unidentified osmotic fraction (UOF) in plasma was derived from measurements of osmolality, Na^+ and Cl^- using UOF = [1- ((Na + Cl)/osmolality)] *100 (Giles et al. 1984).

Results

Condition factor was not different between years for salmon held in the Medway River (Fig. 3). Exposure to Westfield River water reduced condition factor both years but to a greater extent in 1985 than 1986. Limestone treatment restored condition to a level equal to that found in the Medway River fish. Feeding the high-salt diet did not affect condition of fed fish held in Westfield River water.

Plasma osmolality, Na^+, Cl^-, UOF, Ca^{++} (female) and T_3 were depressed in salmon held in the Westfield River in 1985 and 1986 compared to those held in the Medway River (Table 1). Partitioning of the treatment effects showed a significant ($P<0.0375$) trend toward reduced plasma Na^+ with increasing exposure time after 63 d and significantly reduced plasma Cl^- after 111 d exposure (Fig. 2). Salmon held in limed Westfield River water generally had plasma osmolality, Na^+ and Cl^- concentrations similar to or greater than those of fish in the Medway River (Table 1; Fig. 4). However, when spawning first started in 1986 (Fig. 4), plasma Na^+ fell in the group held in limed Westfield River water to values not different from those in fish held in untreated Westfield River water. Liming did not reverse the depressive effects of Westfield River water on plasma Ca^{++} (female) and plasma T_3 (Table 1). Feeding the high-salt diet restored plasma osmolality, partially restored plasma UOF, Na^+, and plasma Ca^{++} (female), but did not alter Cl^-, or plasma T_3 in the Westfield River fish. In addition, exposure to Westfield River water increased salmon plasma levels of K^+ and glucose. These two effects were not reversed by liming or the high-salt diet.

No differences in measured plasma constituents between cage-held and tank-held fish were evident (Table 1).

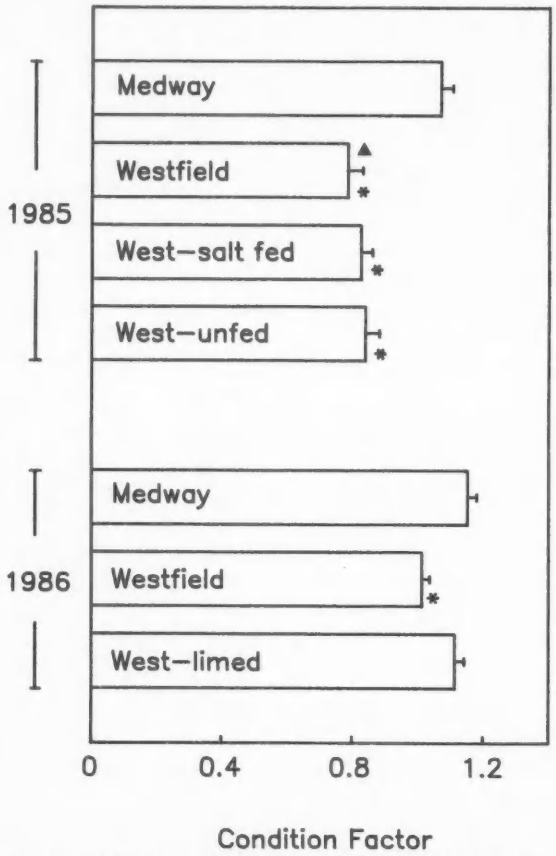

FIG. 3. Condition factor of Atlantic salmon held in the Medway River and the Westfield River in 1985 and 1986. Values represent mean ± SE of 12 fish. *indicates Westfield River means significantly different from the respective Medway River means. ▲ indicates 1985 means significantly different relative to 1986 means.

TABLE 1. Plasma concentrations of osmolality, sodium, chloride, unidentified osmotic fraction (UOF), potassium, calcium, glucose, protein, thyroxine, and triiodothyronine in Atlantic salmon held in the Medway River and the Westfield River in 1985, 1986, and 1987. Values represent mean (SE) of 12 fish (6 male, 6 female) except for calcium which represent mean (SE) of 6 fish. * indicates significantly different from corresponding value in fish from the Medway River ($P < 0.05$). ▲ indicates 1985 means significantly different relative to the corresponding 1986 mean ($P < 0.05$).

Year	Group	Osmolality (mosmol/kg)	Sodium (mmol/L)	Chloride (mmol/L)	UOF** %	Potassium (mmol/L)	Calcium Male (mmol/L)	Calcium Female (mmol/L)	Glucose (mmol/L)	Protein (g/L)	Thyroxine (mmol/L)	Triiodothyronine (mmol/L)
1985	Medway	319 ▲	159	145 ▲	4.7	3.0	2.4	4.0 ▲	9.0	27.5	11.8 ▲	3.8 ▲
		(2)	(1)	(1)	(0.5)	(0.1)	(0.1)	(0.1)	(1.0)	(2.6)	(0.7)	(0.4)
	Westfield	307 *	138 *▲	125 *	14.3 *▲	3.9 *	2.2	2.9 *	25.8 *▲	30.7	9.1 ▲	2.5 *
		(3)	(2)	(2)	(0.8)	(0.1)	(0.1)	(0.4)	(1.6)	(2.3)	(0.6)	(0.3)
	West-salt fed	311	146 *	127 *	12.1 *	3.7 *	2.3	3.3	23.6 *	36.1	9.3	2.3 *
		(3)	(2)	(2)	(0.8)	(0.1)	(0.1)	(0.4)	(1.6)	(2.2)	(0.7)	(0.3)
	West-unfed	304 *	139 *	122 *	14.4 *	3.7 *	2.1	2.8 *	25.2 *	34.4	13.7	2.5 *
		(4)	(3)	(2)	(0.4)	(0.1)	(0.1)	(0.4)	(1.2)	(2.3)	(1.1)	(0.3)
1986	Medway	308	161	136	3.5	2.9	2.2	5.9	7.5	39.8	18.6	8.0
		(3)	(1)	(1)	(0.8)	(0.1)	(0.1)	(0.8)	(1.0)	(5.4)	(1.8)	(0.8)
	Westfield	303	152 *	130 *	7.1 *	3.4 *	1.8	2.6 *	15.4 *	28.6	18.4	2.6 *
		(4)	(3)	(3)	(1.1)	(0.1)	(0.2)	(0.4)	(1.4)	(5.1)	(2.0)	(0.3)
	West-limed	309	161	134	4.1	3.3 *	2.0	2.6 *	11.2 *	32.9	14.1	3.0 *
		(3)	(1)	(1)	(0.9)	(0.1)	(0.1)	(0.3)	(1.0)	(3.8)	(1.2)	(0.4)
1987	Medway caged	303	156	135	5.0	2.8	2.3	3.6	5.4	33.9	14.7	3.9
		(3)	(2)	(2)	(0.6)	(0.1)	(0.2)	(0.3)	(0.3)	(1.6)	(1.6)	(0.3)
	Medway tank	308	158	138	3.9	3.6	2.6	3.4	7.1	38.9	12.1	4.1
		(3)	(2)	(1)	(0.8)	(0.1)	(0.1)	(0.5)	(1.5)	(5.7)	(1.2)	(0.6)

**UOF = $[1-(Na^+ + Cl^-)/\text{osmolality}]*100$.

Salmon held in the Medway River had higher plasma osmolality and Cl^- along with lower plasma Ca^{++} (female), T_4, and T_3 in 1985 relative to values observed in 1986. In the Westfield River, plasma UOF, K^+ and glucose were higher while plasma Na^+ and T_4 were lower in 1985 than in 1986 (Table 1).

Gill morphology of Atlantic salmon was typical of salmonids and similar to rainbow trout (*Oncorhynchus mykiss*) gills described by Evans et al. (1988). Mean thickness of the primary epithelium was 19.5 μm in gills of Medway River fish but was about 26.0 μm in those of Westfield River fish (Fig. 5, 6). Also, in fish exposed to Westfield River water, there was an increased width of the base of the secondary lamellae and a more rugose appearance of the secondary epithelium. The gill responses were evident both years in fish from untreated Westfield River water but no difference between years was apparent in gill morphology. Liming effectively reduced these alterations in gill morphology produced by exposure to Westfield River water. Feeding fish with the high-salt diet had no ameliorative effects on gills of fish held in the Westfield River.

Discussion

Effects of Acidic Westfield River Water

The fundamental toxic mechanism of direct acid stress in the gills is the disturbance of electrolyte regulatory processes (Wood and McDonald 1982). Exposure to acidic water increases ion efflux and inhibits ion influx. Ultimately, death is caused through shifts in secondary fluid volume, hemoconcentration and circulatory collapse (Wood and McDonald 1982). Depressed plasma Na^+ and Cl^- in Atlantic salmon held in Westfield River water agrees with previously observed effects of acidic water on electrolyte balance in Atlantic salmon (Lacroix 1985*b*; Lacroix and Townsend 1987), rainbow trout (*Oncorhynchus mykiss*) (McDonald 1983; McDonald et al. 1983; Giles et al. 1984) and brook trout (*Salvelinus fontinalis*) (Booth et al. 1988).

Because about 90% of ion loss in acid exposed fish occurs at the gills (McDonald 1983), hypertrophied gill tissue likely represented an adaptive response of Westfield River fish to reduce ion permeability. Thickening of primary epithelium increases the distance between the circulatory compartment and the external environment and reduces the surface area of exposed secondary lamellae epithelium (Leino et al. 1987; Evans et al. 1988). Exposure to acidic conditions was related to primary epithelial hyperplasia in a variety of fish species (reviewed by Evans et al. 1988). Measurement of epithelial thickening may represent a sensitive indicator of exposure to acidic environments.

Plasma osmolality was depressed in Atlantic salmon held in Westfield River water, but its reduction was not as great as might have been expected from decreases in Na^+ and Cl^- alone. Changes in plasma osmolality were ameliorated to some extent by increases in undefined osmotic constituents. This undefined osmotic fraction (UOF) represents increased plasma solutes other than Na^+ and Cl^-, probably consists of glucose, amino acids, K^+, and phosphate, acquired from intracellular compartments or the diet to help maintain extracellular fluid balance (Fugelli and Vislie 1982; Milligan and Wood 1982; Tam et al. 1987; Majewski et al. 1990). Similar to what Giles et al. (1984) found for rainbow trout, the UOF was a consistent and sensitive response of Atlantic salmon to acidic Westfield River water.

The major role of glucose in fish during acid stress remains to be defined. Alterations of carbohydrate metabolism towards high circulating glucose levels and gluconeogenesis are consistent responses of fish to acidic conditions (Brown et al.

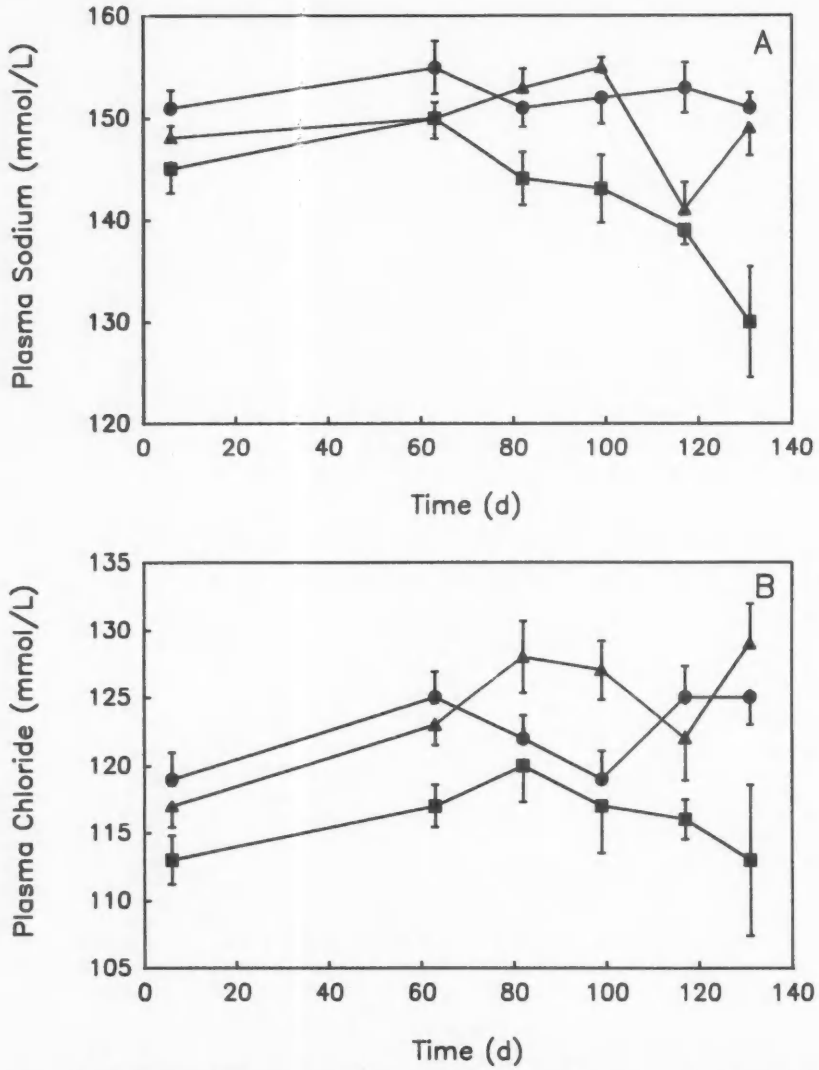

FIG. 4. Concentrations of (A) Na^+ and (B) Cl^- in Atlantic salmon held in the Medway River (●), untreated Westfield River water (■) and limed Westfield River water (▲) in 1986. Points represent mean ± SE of 10 fish sampled repetitively.

1984; Haya et al. 1985; Brown et al. 1986; Scherer et al. 1986; Tam et al. 1987). Because increases in standard metabolic rate are not evident during acute or chronic acid exposure (Ultsch et al. 1980; Tam et al. 1987), it is possible that these fish are not using the high glucose to derive extra energy. This observation supports speculation that glucose plays a more passive osmotic role as a biochemical adaptation to losses of Na^+ and Cl^-. Accordingly, plasma glucose is generally highly correlated with UOF in this and other studies (Brown et al. 1986; Scherer et al. 1986; S. B. Brown unpubl. data).

Data obtained in 1985 are difficult to interpret in terms of acid stress only because the fish were handled and bled 1 or 2 d before our terminal sampling. Although potential responses of plasma ions and glucose might be obscured by the additional acute handling stress, the 1985 data are worthy of comment because fish in both rivers were handled in a similar fashion. However, in 1986 fish were not disturbed for 15 d before final sampling in December. Reductions in Na^+ and Cl^- along with increases in glucose, K^+, and UOF, although less severe, were still evident with the similar exposure period (149 d in 1985 vs. 126 d in 1986) and similar pH differential (0.4 in 1985 vs. 0.5 in 1986).

Acute handling stress increases water and ion permeability of fish gills. Fish held in freshwater respond by a gain in weight, loss of blood electrolytes, and an increase in blood glucose derived from tissue glycogen (Wedemeyer 1972; Mazeaud et al. 1977; Redding and Schreck 1983; Woodward and Strange 1987; Majewski et al. 1990). Atlantic salmon held in the

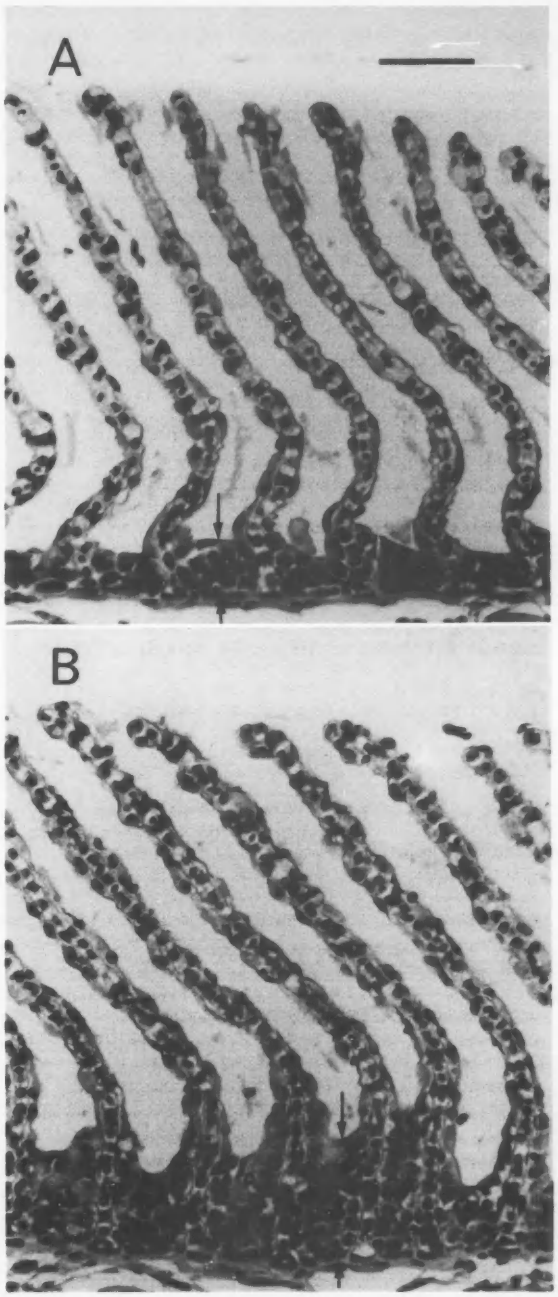

FIG. 5. Paraffin sections (8 μm) of Atlantic salmon gills stained with Harris' hematoxylin and eosin. Gills of fish held in (A) Medway River and (B) untreated Westfield River water. Note thickening of primary lamellae epithelium (←→), and more rugose appearance in fish held in the Westfield River. Bar = 50 μm.

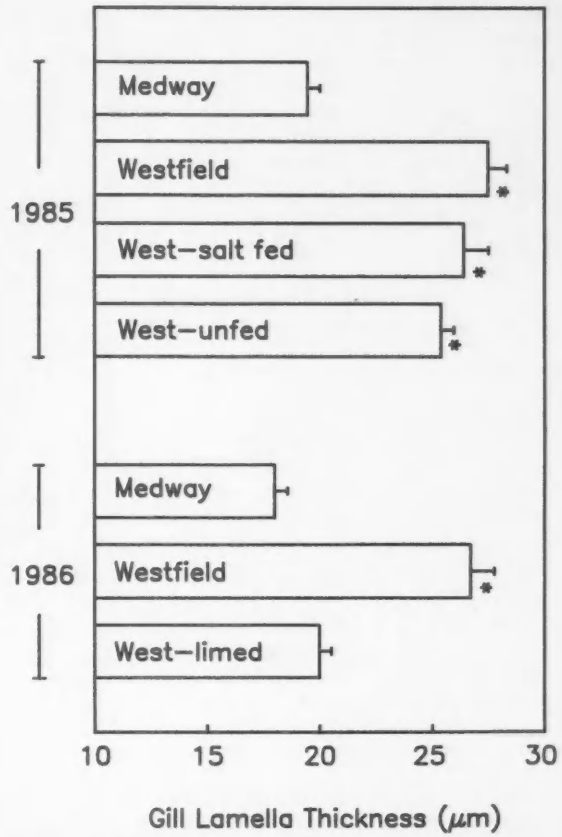

FIG. 6. Epithelial thickness of primary gill lamellae from Atlantic salmon held in the Medway River and the Westfield River, 1985–1987. Values represent mean ± SE of gill measurements from 12 fish. *indicates Westfield River means significantly different from its respective Medway.

Medway River were able to recover from the effects of handling within 24 h. With the exception of plasma Ca^{++} (female), electrolyte constituents were similar to or greater than those found in salmon held in the Medway River in 1986 and to previously reported values considered normal (Byrne et al. 1972). However, plasma Na^{+} and Cl^{-} were more severely depressed in fish from the Westfield River, compared to values found in 1986. Moreover, plasma glucose was extremely high in Westfield River salmon in 1985 relative to 1986. These data support the hypothesis that acid-exposed fish appear more sensitive to additional stresses than non-exposed fish (Barton et al. 1985). The criticisms that acute handling could have altered blood constituents might also be applied to the repetitive sampling regime employed in this study. A better but more costly protocol would involve use of multiple cages for each treatment and sampling all individuals from a single cage at each sampling time or by samping the fish without replacement. Nevertheless, Atlantic salmon held in Medway River water in 1985 maintained their blood ions and glucose near those observed the following year. Fish exposed to Westfield River water could not maintain electrolyte balance and their ability to ionoregulate must be considered impaired.

In 1987, fish held in cages or in tanks had similar levels of the measured plasma constituents. Thus, it appeared unlikely that the altered levels of plasma ions, UOF, glucose, and T_3

between Medway River fish and Westfield River fish were due to the different holding conditions in 1986.

Depressed plasma Ca^{++} was also reported in female white suckers (*Catostomus commersoni*) from acidic, low calcium environments (Lockhart and Lutz 1977; Weiner et al. 1985). Because reduced calcium levels occurred only in female fish, it seems logical that the reduced calcium could have been associated with abnormal steroid metabolism and egg development (Sangalang et al. 1990). Estradiol induces synthesis and secretion of vitellogenin by the liver in female fish (Whitehead et al. 1983). The vitellogenin is transported by the bloodstream as a complex with Ca^{++} to the ovary where it is taken up into oocytes. Compared to female fish from the Medway River, fewer females held in the Westfield River in 1986 spawned, and those which did had smaller (0.3 μm) and fewer (22 per 100 g body wt.) eggs (Sangalang et al. 1990). Altered vitellogenin production itself may result in low plasma vitellogenin and low Ca^{++} levels in female fish (Parker and McKeown 1987; Mount et al. 1988). Consistent with limited Ca^{++} availability, there is evidence of Ca^{++} reductions in vertebral bones from these salmon (Majewski et al. 1990). Exposure of rainbow trout and brown trout (*Salmo trutta*) to acidified soft water caused net losses of body ions including Ca^{++} (Höbe 1987; Reader and Morris 1988). Low pH also reduced accumulation of Ca^{++} and ossification in larval salmonids (Nelson 1982; Peterson and Martin–Robichaud 1986). Because plasma Ca^{++} was not affected in male salmon, our results suggest that female fish could maintain normal Ca^{++} levels but could not meet added requirements for vitellogenin production. Changes in normal plasma Ca^{++} appeared more related to Ca^{++} availability in the environment (Rodgers 1984; Bendell-Young and Harvey 1986) or the diet (Sadler and Lynam 1986).

Plasma T_4 and T_3 levels in fish held in the Medway River were similar to those previously reported for juvenile Atlantic salmon (Rydevik et al. 1984; McCormick et al. 1987). Reduced plasma T_3 in Atlantic salmon held in the Westfield River corroborate findings by Brown et al. (1984) for acid-stressed rainbow trout. There was no evidence for a corresponding increase in plasma T_4 levels described for rainbow trout (Brown et al. 1984, 1986) and brown trout following acid exposure (Edwards et al. 1987). In contrast, thyroid hormone levels in smolting Atlantic salmon showed no clear effects due to low pH (Saunders et al. 1983). Elevated plasma T_3 has been implicated in somatic growth (Donaldson et al. 1979; Higgs et al. 1982). Consequently, reduced T_3 levels in salmon from the Westfield River could have been indicative of poor condition and weight loss relative to fish held in the Medway River (Sangalang et al. 1990).

Plasma T_4 and T_3 did not show expected increases in fish fed the Co-op or the high-salt diet relative to fish deprived of food in 1985. Generally, indices of thyroid function increase in proportion to quantity and quality of food supply (reviewed by Eales 1988). However, the thyroid system also responds to both acute and chronic stress (Eales 1979) and handling effects along with chronic acid stress likely obscured potential dietary differences. Responses to the handling stress may also explain lower levels of T_3 and T_4 for salmon held in the Medway River in 1985 compared to those found in Medway River fish in 1986.

Mitigation

Liming increases pH and Ca^{++} concentration of acidic waters (Skogheim et al. 1987). Liming Westfield River water reduced much of the physiological stress. Plasma Na^+ and Cl^- levels and epithelial thickness of primary gill lamellae were similar to those found in Medway River salmon. Similarly, plasma Cl^- and survival of Atlantic salmon were increased by liming an acidic Norwegian River (Rosseland et al. 1986). Increased condition and reduced weight loss in West-limed group imply that the fish were in a more healthy state than fish exposed to Westfield River water. Liming was not totally effective because plasma glucose, T_3 or Ca^{++} (female) were not restored to levels found in Medway River salmon. However, the West-limed group were exposed to untreated Westfield River water for 35 d prior to lime-treated water. Simply returning water pH and Ca^{++} to values found in the Medway River may not be sufficient to allow full recovery from initial changes caused by the exposure to low pH or that another factor contributes to the toxicity of limed Westfield water. The presence of an unknown toxicant in Westfield River water or particulates formed during raking could represent possible candidates. Lime treatment reduced the physiological stress created by exposing salmon to the acidic Westfield River, but it represented a compromise for the fish previously exposed to the acidic conditions. To evaluate the effectiveness of lime treatment for protection of unexposed salmon further experiments are required.

The protective effect of salt feeding could not be fully evaluated because of acute handling stress imposed shortly before the last sampling period in 1985. Plasma osmolality and ions in fish fed the high-salt diet were closer to levels found in Atlantic salmon from the Medway River than levels in the fish held in the Westfield River, but the same extent of gill hyperplasia was evident. Apparently, salt feeding did not protect fish from acid stress; if it had, plasma constituents and gill morphology of Westfield River fish would have been similar to those found in Medway River fish.

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Responses of Kidney, Liver, Muscle, and Bone in Atlantic Salmon (*Salmo salar*) to Diet and Liming in Acidic Nova Scotia Rivers

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Two-year old sexually maturing Atlantic salmon (*Salmo salar*) were held in the Medway (pH range 5.2–5.6) and Westfield (pH range 4.7–5.2) rivers of Nova Scotia for 149 and 126 d respectively, in successive years (1985 and 1986). Exposure to Westfield river water resulted in a depletion of renal and hepatic acid-soluble thiol (AST) and of renal ascorbic acid (AsA) reserves in both years. Liming, or the feeding of a high-salt (3.0% NaCl) diet, failed to maintain completely these reserves at levels found in Medway river salmon. In 1986 declines in bone (Ca and P) and muscle (Na and K) electrolytes were coincidental to elevations in liver glycogen, suggesting that gluconeogenesis was an adaptive mechanism in response to the ionoregulatory effects associated with acidic and low ambient calcium conditions. The addition of lime to Westfield river water restored muscle electrolyte levels, but had no effect on depleted bone Ca and P levels.

Des saumons de l'Atlantique (*Salmo salar*) en voie de maturation, âgés de deux ans, ont été placés dans les rivières Medway (pH 5,2–5,6) et Westfield (pH 4,7–5,2) (Nouvelle-Ecosse) pendant respectivement 149 et 126 j, au cours des années 1985 et 1986. L'exposition à l'eau de la rivière Westfield s'est traduite par un épuisement des réserves rénale et hépatique de thiol acido-soluble (AST), et des réserves d'acide ascorbique rénal, au cours des 2 ans. Le chaulage, ou un régime à forte teneur en sel (3.0% NaCl), n'a pas permis de maintenir complètement ces réserves au niveau relevé dans les saumons de la rivière Medway. En 1986, les baisses d'électrolytes dans les os (Ca et P) et dans les muscles (Na et K) coïncidaient avec les augmentations de glycogène hépatique, ce qui porte à croire que la gluconéogenèse était un mécanisme d'adaptation aux effets ionorégulateurs associés à des teneurs ambiantes acides et faibles en calcium. L'addition de chaux à l'eau de la rivière Westfield a rétabli le taux d'électrolytes dans les muscles, mais n'a pas eu d'effet sur la diminution du Ca et du P dans les os.

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Atlantic salmon (*Salmo salar*) populations have either declined or disappeared entirely from a number of rivers in southwestern Nova Scotia due to acidification of these soft and poorly buffered waters (Farmer et al. 1980; Watt et al. 1983). Minimum pH in these rivers occurs after autumn rains and increased water flow (Lacroix and Kan 1986). In recent years field studies have been conducted to investigate changes in the physiology and biochemistry of Atlantic salmon affected by environmental acidification. Salmon exposed to a pH range of 4.9–5.3 in the Westfield river experienced loss of ionoregulatory control (Lacroix 1985*a*, *b*; Lacroix and Townsend 1987) and an inhibition in reproductive capacity (Freeman et al. 1983*a*, *b*; Freeman and Sangalang 1985). These observations are similar to dose–response relationships established in the laboratory for other salmonids (Wood and McDonald 1982; Brown et al. 1984; Giles et al. 1984).

This field study is part of a collaborative, multidisciplinary effort attempting to relate biochemical, physiological, and histopathological changes in individual fish to potential population effects. An integrated approach has been proposed (National Research Council of Canada 1985) as a requirement for assessing fish health in response to toxic chemicals. The more traditional biological monitoring of populations does not provide for early detection of a gradually deteriorating ecosystem or establish cause-effect relationships.

The objective of this study was to validate in the field biochemical parameters known to be indicators of physiological stress in fish (Wedemeyer and McLeay 1981; Hamilton et al. 1981; Thomas et al. 1982a, 1982b; Wood and McDonald 1982; Lee et al. 1983; Giles et al. 1984; Wofford and Thomas 1984) in order to determine their utility as early warning indicators of the sublethal effects of environmental acidification and to assess their responsiveness to mitigation measures in the field. For this purpose, 2-yr-old, sexually maturing Atlantic salmon were held in the Medway (pH range 5.2–5.6) and Westfield (pH range 4.7–5.2) rivers in 1985 and 1986 for 149 and 126 d, respectively. The more acidic Westfield river was used to study the potential ameliorative effects of feeding a high-salt (3.0% NaCl) diet in 1985, and lime addition to the water in 1986. Biochemical responses analyzed as indicators of physiological dysfunction/compensation were renal and hepatic ascorbic acid (AsA) and acid-soluble thiol (AST) content, liver glycogen, muscle electrolytes (Na and K) and bone electrolytes (Ca and P).

Materials and Methods

Experimental Sites

Holding cages and tanks were constructed in the Medway River and a tributary, the Westfield River (Sangalang et al.

1990). The sites were located in the upland region of southwestern Nova Scotia (Brown et al. 1990, their figure 1). The pH of each river was recorded daily. Water was collected in mid-December and total aluminum, cadmium, calcium, copper, iron, magnesium, manganese, zinc, and dissolved organic carbon determined by standard methods at the Freshwater Institute Chemistry Laboratory.

The pH (range 4.7–5.2) of the Westfield River was 0.3 to 0.6 lower than the pH (range 5.2–5.6) of the Medway River (Brown et al. 1990, their figure 2). In 1986, liming restored pH (range 5.1 to 5.9) of Westfield River water to values observed in the Medway River. Calcium levels averaged 25 μmol/L in the Medway River and 17 μmol/L in the Westfield River. Liming Westfield River water increased calcium levels to 32 μmol/L. Other measured constituents were not altered by lime treatment and were similar to those described previously for these rivers (Watt et al. 1983; Lacroix 1985a, b; Lacroix and Townsend 1987).

Experimental Fish

Sexually maturing 2-yr-old Atlantic salmon (River Phillip stock), weighing 600–1200 g, were purchased from a local fish farm (Ocean Farms Ltd., Upper Tantallon, N.S.) in 1985 and 1986. After acclimation to freshwater, the fish were initially transported to the Medway River and placed in floating fish cages (3.7 m × 2.2 m × 1.8 m deep, 25 mm mesh netting) (Freeman and Sangalang 1985). After a minimum acclimation of 1 wk, fish were tagged, grouped, and some transported to the Westfield River as outlined above.

Post-spawned fish mortality in the more acid Westfield River was 30%. There were no mortalities of post-spawned fish held in the Medway River or in limestone treated Westfield River water.

Experimental Protocol

1985

On July 15, 1985, the fish were separated into four groups (Medway, Westfield, West-salt fed, and West-unfed) of 46 fish having equal numbers of each sex. The Medway group remained caged in the Medway River and was fed a commercial trout grower (Co-op diet, 0.3% NaCl). The other three groups were placed into cages in the Westfield River. The Westfield River groups were treated as follows: the Westfield group was fed the Co-op diet; group West-salt was fed a high salt diet (3.0% NaCl); the West-unfed group was not fed. All other details on dietary constituents are given in Sangalang et al. (1990). The fish were fed once daily until mid-October and once every other day thereafter.

1986

On August 6, 1986, salmon were divided into three groups of 40 fish designated Medway, Westfield, and West-limed. The Medway group was held in a floating cage in the Medway River. The other groups were moved to tanks located in a branch of the Westfield River (Sangalang et al. 1990). Each tank received river water at 23–30 L/min. Starting September 10, 1986 the West-limed group received river water that was passed through and over crushed limestone which was raked daily. The Westfield group received untreated Westfield River water. All fish were fed the Co-op diet daily.

Total exposure times of Atlantic salmon to Westfield river water were 149 d and 126 d in 1985 and 1986, respectively. In 1986, the West-limed group were exposed to Westfield River water for 35 d before receiving limestone-treated water for the last 91 d of the experiment.

Blood Sampling

Before the terminal sample described below, the fish were lightly anesthetized with 35 mg/L tricaine methanesulphonate (TMS), weighed, measured, and 1 mL of blood removed at the beginning of the experiment and 0.5 mL at 2- to 3-wk intervals (Sangalang et al. 1990).

Unfortunately, in 1985 the fish were handled and bled only 1 or 2 d before our terminal sampling. In the following year, a 2- to 3-wk recovery period was allowed before terminal sampling.

After the final bleeding for a reproductive hormone study (Sangalang et al. 1990) in early December each year, 12 post-spawned salmon from each group (6 males, 6 females) were sacrificed by immersion in pH-neutralized TMS (200 mg/L). Within a few minutes of being immobilized, the fish were bled from the caudal vessels using ammonium-heparinized 10-mL syringes fitted with 18-gauge needles. Plasma was immediately separated by centrifugation and frozen on dry ice in sterile 10-mL polypropylene tubes. Upon return to the laboratory plasma samples were stored at −120°C until analyzed.

Tissue Sampling

Epaxial muscle, liver, posterior kidney, and vertebral bone samples were removed from each fish within 15 min following blood sampling. Tissues were placed in plastic bags and quick frozen on dry ice. Samples were returned to the laboratory (2–4 d) and subsequently stored at −120°C until analyzed.

Biochemical Analyses

1985 and 1986

Posterior kidney and liver acid-soluble thiol (AST) content was determined using the 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) method of Moron et al. (1979). Ascorbic acid (AsA) content of posterior kidney and liver was analyzed using Folin–Ciocalteu phenol reagent in an acidic pH (Jagota and Dani 1982). Liver glycogen was measured using the phenol/sulfuric acid method of Montgomery (1957), and liver and kidney protein were measured using a modification of the micro-Lowry method (Peterson 1977).

1986

A section of vertebral bone (posterior to the head and anterior to the dorsal fin) was taken from each fish. Spines and soft tissue were removed leaving only the centra. These centra were split into two fractions and dried to a constant weight at 100°C in a vacuum oven for 24 h. One fraction was hydrolyzed at 115°C in 5 mL of 6N HCl for 16 h (Hamilton et al. 1981) and used to measure bone calcium (Gitelman 1967), and phosphorus (Fiske and Subbarow 1925). Collagen was isolated from the other fraction by the method of Flanagan and Nichols (1962). This collagen was weighed and hydrolyzed at 115°C, and used for the determination of hydroxyproline (Woessner 1961).

Epaxial muscle was removed from the area just below the base of the dorsal fin extending to the lateral line. A subsample of approximately 2 g was weighed, dried under vacuum at 95°C for 24 h, and reweighed to determine tissue water content. Muscle electrolyte concentrations were measured by homogenizing 1.0 g of muscle with 3.0 mL of distilled water for 30 s

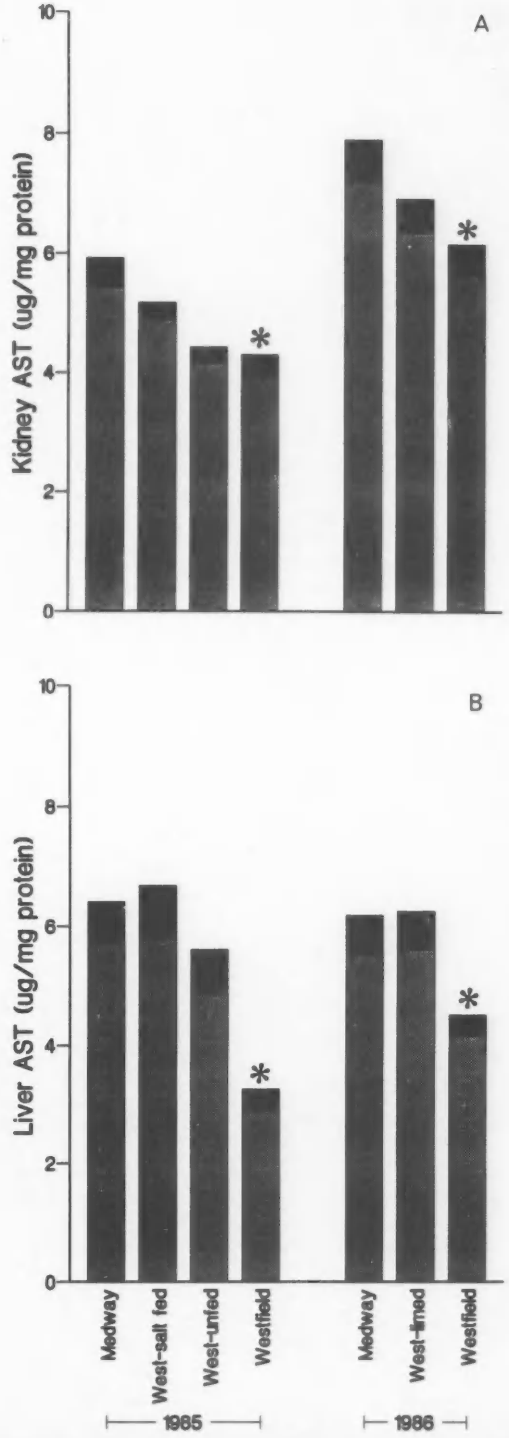

FIG. 1. Concentration (μg/mg protein) of acid-soluble thiols (AST) in (A) posterior kidney and (B) liver of Atlantic salmon held in the Medway and Westfield rivers in 1985 and 1986. Values represent mean (hatched bar) ± SEM (solid bar) of 12 fish (6 male and 6 female). Westfield River groups significantly different from the Medway River group in the same year are indicated (* = $P < 0.05$; ** = $P < 0.1$).

with a Willems polytron. Homogenized samples were capped, stored for 4 d at 4°C, and subsequently centrifuged at 2000 × *g* for 20 min (Eddy and Bath 1979). The supernatant was analyzed for sodium and potassium using an IL 943 flame photometer.

Statistical Analyses

Bartlett's test for homogeneity of variance was applied to the data and, where required, data were transformed according to Taylor's power law (Southwood 1978) to obtain uniform variances. For clarity of presentation, untransformed arithmetic means and standard errors are given in the results section. Analyses of variance were computed using the GLM procedure of the Statistical Analysis System (SAS Institute Inc. 1982). A three-way factorial arrangement using year, group, and sex was applied to test for significant differences in fish from the terminal sampling in December. Differences between group means were examined using Tukey's Studentized Range test. A probability level of ≤ 0.05 was considered significant.

Results

Acidification Responses (1985 and 1986)

Posterior kidney and liver AST content was significantly lower in salmon from the more acidic Westfield river in both 1985 and 1986 (Fig. 1A, 1B). In 1985, mean kidney and liver AST values in Westfield salmon were 75% and 53%, respectively, of those observed in Medway river salmon and in 1986 these values were 71% and 75%, respectively.

Posterior kidney AsA content was also significantly lower in Westfield river salmon in both years of this study (Fig. 2A) with mean values (for 1985 and 1986, respectively) of 72% and 64% of those observed in Medway river salmon. A similar trend was observed for liver AsA content in 1986 (Fig. 2B). Female salmon from both rivers had lower levels of liver AsA than males (41–76%) in both years and lower levels of posterior kidney AsA in 1986 (68–92%).

Liver glycogen content was significantly higher in Westfield river salmon in 1986; the mean value being 163% of that observed in Medway river salmon (Fig. 3A). Both female and male fish responded in a similar manner (Fig. 3B, 3C). In 1985, however, male salmon from the Westfield river, which were not fed the high-salt diet, had significantly lower glycogen levels than their Medway counterparts (Fig. 3B). Female salmon from both rivers had much lower levels of liver glycogen than their male counterparts (Fig. 3B, 3C) in both years of this study.

Bone calcium and phosphorus levels were significantly lower in fish from the Westfield River in 1986 (Fig. 4A, 4B). There were no treatment differences associated with the organic fraction (i.e. collagen and hydroxyproline) of bone.

Muscle electrolytes (Na^+ and K^+) declined in response to the lower pH in the Westfield River fish in 1986 (Fig. 5A, 5B).

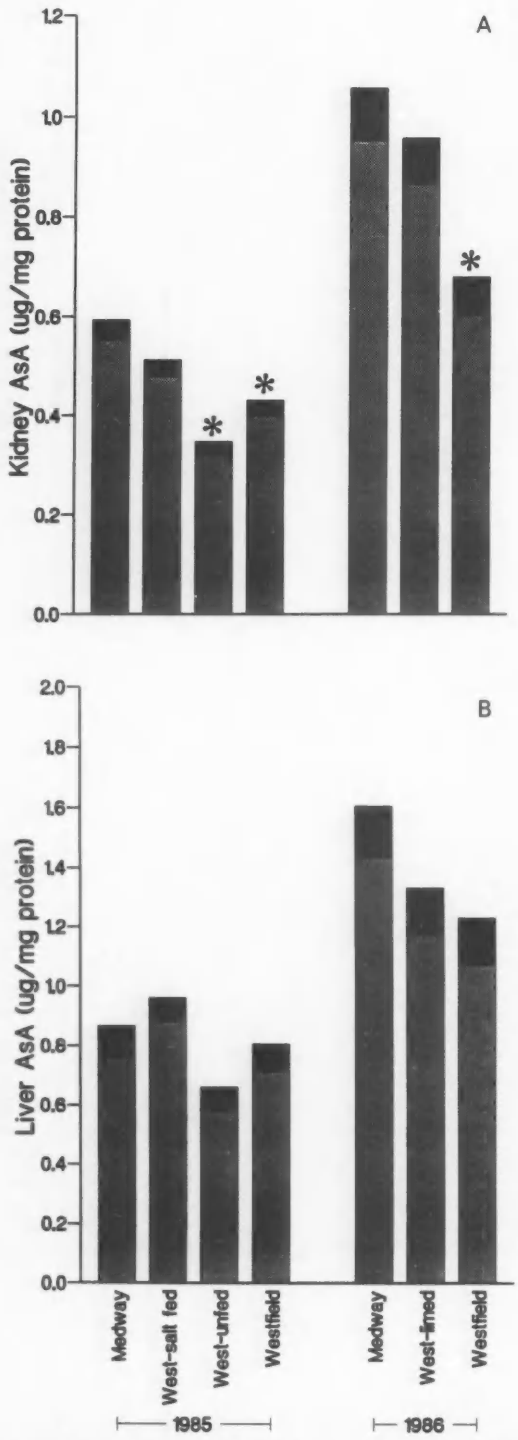

FIG. 2. Concentration (μg/mg protein) of ascorbic acid (AsA) in (A) posterior kidney and (B) liver of Atlantic salmon held in the Medway and Westfield Rivers in 1985 and 1986. Values represent mean ± SEM of 12 fish (6 male and 6 female). Statistical symbols are described in Fig. 1.

Mitigation Responses

High-salt diet (1985)

Atlantic salmon fed a high-salt (NaCl) diet exhibited some improvement in terms of adapting to the lower pH of the Westfield River. Mean kidney and liver [AST] (Fig. 1A, 1B) in these fish were elevated relative to Westfield fish fed either standard commercial trout pellets or no food. Kidney and liver [AST] were within 90 and 101%, respectively, of the value for Medway River fish fed the standard commercial diet, and kidney [AsA] was 87% of this value (Fig. 2A).

Liver glycogen in Westfield River male salmon fed the high-salt diet was significantly elevated (approx. 3X) above the levels in fish fed either the standard diet or no food (Fig. 3B). Glycogen levels in salt-fortified female salmon were much lower than those found in male fish (approx. 15%) and there were no significant differences due to diet (Fig. 3C).

Liming (1986)

Liming the Westfield River water modified the biochemical response of salmon to acidification. Kidney and liver AST levels in Westfield-limed fish were 88 and 102%, respectively, of the values found in Medway River fish (Fig. 1A, 1B). Kidney AsA levels in Westfield-limed fish were within 91% of those found in Medway River fish (Fig. 2A), and liver glycogen levels approximately equalled those found in Medway fish (Fig. 3A). A similar response to liming was observed in muscle sodium (Fig. 5A) and potassium (Fig. 5B). The mean values for these elements were 105 and 102%, respectively, of those found in Medway River salmon. Liming did not restore bone calcium (Fig. 4A) or phosphorus (Fig. 4B) levels which were 77 and 66%, respectively, of those observed in Medway River salmon.

Discussion

Acidification

Lower acid-soluble thiol (AST) levels were observed in post-spawning salmon held in untreated Westfield river water in both years of this study. The DTNB method for the analysis of AST content in fish tissue is predominantly a measure of metallothionein (MTN) and glutathione (GSH) (Wofford and Thomas 1984). These two compounds are of critical importance in detoxification processes in fish. A major concern under conditions of environmental acidification is the mobilization of heavy metals into the water column. Increases in unbound Cd, Zn, and Cu can induce the production of MTN which binds up to 90% of the metal accumulated in fish (Noël-Lambot et al. 1978). Thomas et al. (1982b) observed a dose and time-dependent elevation of AST in liver and posterior kidney in the striped mullet (*Mugil cephalus*) in response to a 6-wk Cd exposure. This elevation was found to be due mainly to increased [MTN] within the first 4 wk of exposure followed by elevations in [GSH] after 4 wk (Wofford and Thomas 1984). Lead, which does not induce MTN synthesis, increased GSH levels throughout a 6 wk exposure presumably by stimulating the activity of the enzymes for GSH synthesis (Thomas and

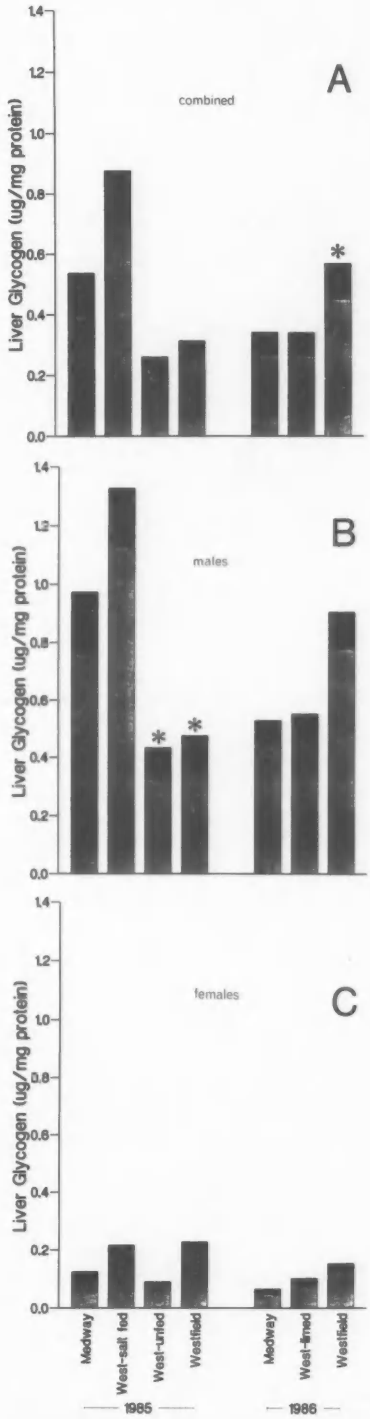

FIG. 3. Concentration (μg/mg protein) of liver glycogen in Atlantic salmon (A) males and females ($n = 12$), (B) males ($n = 6$), and (C) females ($n = 6$) held in the Medway and Westfield rivers in 1985 and 1986. Values represent mean ± SEM. Statistical symbols are described in Fig. 1.

Juedes 1985). In addition, GSH is a substrate or co-factor for a variety of enzymes used for the detoxification and excretion of xenobiotics and their metabolites. The increased utilization of GSH in both mammals and fish exposed to some organics (i.e. monochlorobenzene, bromobenzene, diethyl maleate) results in a depletion of tissue reserves (Chasseaud 1976; Dalich and Larson 1980; Wong and Klaassen 1981). The declines in AST observed in Westfield (untreated) River salmon are likely due to lower levels of GSH, because no changes in renal or hepatic MTN in response to acidification were observed (L. J. Wesson, unpubl. data). The decrease in AST observed here may indicate the involvement of GSH in the detoxification and excretion of unknown xenobiotics, or that GSH is being utilized as substrate for GSH peroxidase (Wendel 1988).

Teleosts, with the exception of some cyprinids, have little or no ability to synthesize ascorbic acid (AsA) as evidenced by a lack of L-gulonolactone oxidase activity (Thomas et al. 1985; Chatterjee 1973). These fish, therefore, are dependent on dietary sources to maintain tissue stores of this vitamin. This dietary requirement has been demonstrated in Atlantic salmon (*Salmo salar*) (Poston and Combs 1979) and rainbow trout (*Oncorhynchus mykiss*) (Sato et al. 1978). Although the biochemical function of AsA in fish is not fully understood, its demonstrated essentiality in vital physiological processes such as ionoregulation (Thomas 1984) and in the maintenance of membrane integrity (Cowey et al. 1985) makes it an important indicator of environmental stress. Fish demonstrate an increased tolerance to a variety of pollutants (PCB, DDT, cadmium, and toxaphene) when tissue stores of AsA are high (cf. Halver 1985), and adequate levels of this vitamin are required for detoxification processes such as the induction of mixed-function oxygenases (Zannoni et al. 1982; Mayer et al. 1978).

Nova Scotia Atlantic salmon are likely to encounter two critical periods in their life cycle when tissue stores of AsA are lowest; as swim-up fry (Cowey et al. 1985) in May and as post-spawning adults in December. Agrawal and Mahajan (1980) found the kidney AsA levels in the carp (*Notopterus notopterus*) were lowest just after spawning. The spawning period of Atlantic salmon is of major ecological importance with respect to environmental acidification in southern Nova Scotia because it follows shortly after autumn rains, when pH levels in these salmon rivers are minimal (Lacroix and Kan 1986). The post-spawning Atlantic salmon sampled from the Westfield River early in December of 1986 had lower posterior kidney AsA, bone Ca and P, and muscle Na and K. Posterior kidney AsA appears to be sensitive to environmental acidification as indicated by declines in both 1985 and 1986, but the relationship to ionoregulation and Ca metabolism remains unclear. In 1985, the decline in AsA was likely related, in part, to an acute handling stress (involving the anterior kidney as well), activating the pituitary-interrenal axis, resulting in elevated plasma cortisol levels (Wedemeyer 1969; Thomas and Neff 1984; Brown et al. 1984) and hyperglycemia observed in these fish (Brown et al. 1990). This was because the fish had been handled and blood samples had been taken within the 2 d prior to terminal sampling. This handling, in addition to the stress associated with acid exposure, induced an acute stress response

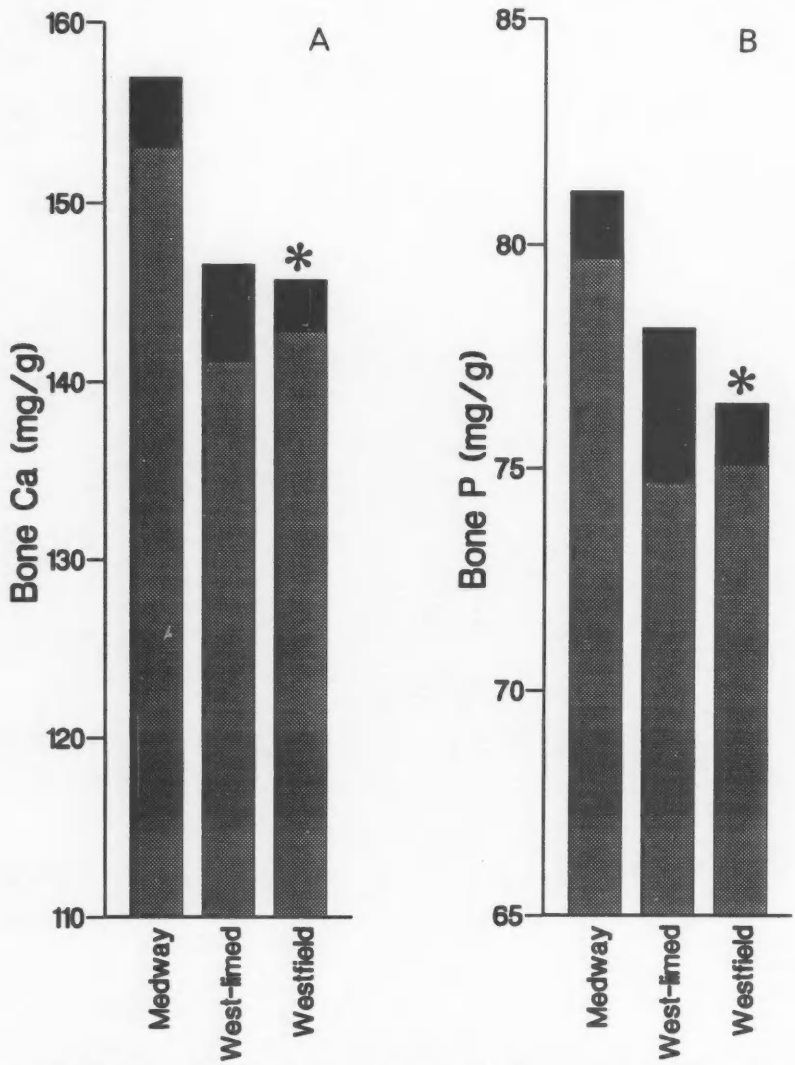

FIG. 4. Concentration (mg/g dry wt.) of (A) calcium and (B) phosphorus in vertebral bone of Atlantic salmon held in the Medway and Westfield rivers in 1986. Values represent mean ± SEM of 12 fish (6 male and 6 female). Statistical symbols are described in Fig. 1.

resulting in a depletion of liver glycogen (in males only) similar in magnitude to that observed in rainbow trout following exercise (Parkhouse et al. 1988). The following year, fish were not handled for 2-3 wk prior to terminal sampling and liver glycogen was elevated. The higher glycogen levels found in these fish in 1986 may be an adaptation to long-term acidification whereby gluconeogenesis occurred to maintain osmotic pressure by increasing plasma glucose to compensate for electrolyte loss. A similar increase in liver glycogen was observed in rainbow trout exposed to pH 4.2 and 4.8 for 14 d (Lee et al. 1983). During chronic exposure to acid, a potentially contributing factor to hyperglycemia may be the catabolism of AsA to glucose via the 2,3-diketo-L-gulonate to D-xylulose pathway. This pathway is thought to exist in the guinea pig, which is also dependent in exogenous AsA to maintain tissue stores of this vitamin (Naito 1980). A slow rate of AsA synthesis from ^{14}C glucose has been shown in trout (Hilton et al. 1979a, b).

In both years of this study, female liver glycogen stores were severely depleted relative to males from both the Medway and Westfield rivers, reflecting the female's increased energy requirement for oocyte development. Although hepatic gluconeogenesis is the primary source of plasma glucose, the relative demand on female salmon AsA stores would be greater if AsA catabolism was also required to maintain plasma glucose levels during chronic acidification. This process would limit the amount of AsA available for oogenesis. The lower tissue stores of AsA observed in female salmon in this study are also

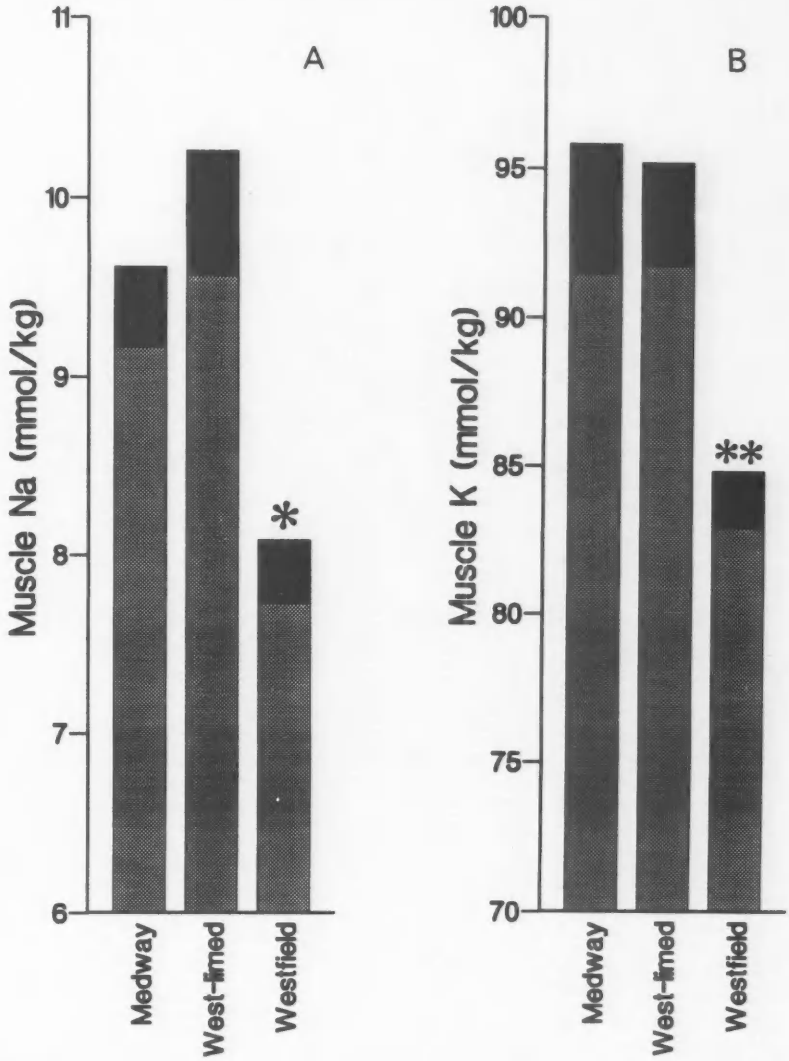

FIG. 5. Concentration (mmol/kg) of (A) sodium and (B) potassium in muscle of Atlantic salmon held in the Medway and Westfield rivers in 1986. Values represent mean ± SEM of 12 fish (6 male and 6 female). Statistical symbols are described in Fig. 1.

likely due to the incorporation of AsA into developing ovarian tissue, where it is involved in steroidogenesis associated with the deposition of egg yolk (Seymour 1981; Sandnes and Braekkan 1981).

The requirement of AsA for Ca absorption and utilization in fish may be a factor in this study in view of the lower levels of posterior kidney AsA observed in Westfield river salmon. Mahajan and Agrawal (1980) reported that an experimentally induced AsA deficiency resulted in decreased absorption and utilization of Ca in the freshwater snake-head (*Channa punctatus*), as indicated in decreased Ca uptake by the gill, bone, muscle, and skin. This resulted in the mobilization of Ca from the bone to maintain Ca homeostasis (Mahajan and Agrawal 1979). Siddiqui (1967) reported a seasonal correlation between AsA and Ca content of various tissues in the green snake-head (*Ophicephalus punctatus*).

The bone demineralization observed in Westfield River salmon in 1986 was similar to that reported by Fraser and Harvey (1982), who found decreased centrum Ca (16%) in resident white suckers (*Catostomus commersoni*) captured in George Lake (pH 4.65; Ca = 2.7 mg/L; DOC = 1.4 mg/L). Demineralization in response to acidification and low ambient Ca levels had previously been demonstrated in laboratory studies with rainbow trout. Holeton et al. (1983) reported that trout lost Ca at a rate of 7.25 $\mu mol \cdot kg \cdot h^{-1}$ when exposed to pH 4.0. Also McDonald and Wood (1981) observed major increases in renal excretion of Ca, phosphate, and K in acid-stressed trout. Bendell–Young and Harvey (1986) suggested

that the reduced Ca:P ratio observed in the bone of white sucker from George Lake (pH 4.8) was attributable to the increased metal concentrations which occur in surface waters coincident with lake acidification. Increased ambient metal loads, however, are not a factor in the Medway or Westfield Rivers since the higher organic content (>10 mg/L DOC) of these rivers would serve to chelate any available metals (Lacroix and Kan 1986; Clair and Freedman 1986; Clair and Komadina 1984). Lacroix and Kan (1986) found that virtually all total extractable Al in the Medway and Westfield rivers in 1985 was non-exchangeable and bound to dissolved organic matter, a form which is much less bioavailable and toxic.

In 1986, muscle electrolyte losses (Na^+ and K^+) in Westfield River salmon accompanied the plasma electrolyte losses reported by Brown et al. (1990). These results corroborate findings by Lacroix and Townsend (1987) and generally agree with laboratory acidification studies which indicate that plasma ions are lost through the gills. An osmotic and ionic gradient occurs between the extracellular and intracellular fluid compartments resulting in water entry into the cellular compartment and electrolyte flux in the opposite direction (Wood and McDonald 1982).

Mitigation

Mitigation measures taken to offset the effects of acidification were of limited value. The high-salt (3% NaCl) diet utilized in 1985 only partially maintained posterior kidney and liver AST and kidney AsA near levels observed in Medway River salmon. Liver glycogen levels in these fish were actually higher than those observed in Medway fish and this response was similar to that observed in Westfield salmon in 1986. It appears that fish fed the high-salt diet did not respond to the handling stress described previously but did respond to the chronic acid stress because they maintained gluconeogenesis as indicated by increased liver glycogen and the maintenance of elevated plasma glucose levels (Brown et al. 1990). Wedemeyer (1972) observed a partial alleviation of handling stress in coho salmon (*Oncorhynchus kisutch*) and steelhead trout (*O. mykiss*) by increasing either ambient NaCl or Ca levels.

The increase in ambient Ca and pH associated with liming (Skogheim et al. 1987) was of some benefit to Westfield River salmon. Rodgers (1984) has previously observed a positive correlation between growth rate and ambient [Ca] in brook trout (*Salvelinus fontinalis*). In 1986 posterior kidney and liver AST and kidney AsA, as well as liver glycogen levels were maintained at levels near those observed in Medway salmon. Also, muscle Na^+ and K^+ levels in Westfield (lime treated) salmon equalled or exceeded levels found in Medway River salmon. The increase (approx. 2×) in ambient Ca probably decreased the net efflux of these ions from the extracellular fluids by decreasing gill permeability, thus conserving intracellular fluid ions. Similar results were found by Wood and McDonald (1982). However, the failure of liming to maintain Ca and P metabolism in Westfield River salmon may be of significance with respect to survival and repeat spawning in limed rivers. Parker and McKeown (1987) have demonstrated that low pH (5.0) has a tendency to decrease total plasma calcium and alkaline-labile phosphate (vitellogenin) in rainbow trout and that bone demineralization occurs in these fish in water with low Ca content (2.1 mg/L). They suggest that low plasma Ca levels during vitellogenesis may be due to an increased loss of Ca from the body. It is therefore important to know how long it takes Atlantic salmon to replenish tissue stores of Ca after low pH and Ca exposure to judge the potential for survival and repeat spawning.

In 1986, Westfield River salmon were exposed to untreated, low pH water for 35 d prior to lime addition and the Ca and P losses likely incurred during this period were not recovered after subsequent lime treatment for 91 d. These ion losses may have been curtailed had pre-exposure to low ambient pH not occurred. However, it is likely that feral migrating Atlantic salmon would encounter acidic conditions prior to reaching suitable spawning grounds. Other factors which confound an accurate assessment of the ameliorative effects of liming under natural environmental conditions include the possibility of the presence of unknown toxicants or the fine particulate matter associated with raking of the limestone.

In conclusion, it appears that long-term environmental acidification in soft water would compromise the potential for successful reproduction and recruitment by Atlantic salmon due to the cost of maintaining homeostasis and the failure of mitigative measures to restore molecular components critical to reproductive success.

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Morphometric Effects of Low pH and Limed Water on the Gills of Atlantic Salmon (*Salmo salar*)

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Gills from adult Atlantic salmon held in water from an acid river (Westfield River, Queens County, Nova Scotia; mean pH 4.8) were compared to gills from fish held in the same water treated with limestone (mean pH 5.5) and gills from fish held in a nearby control river (Medway River; mean 5.4). Morphometric analysis showed that fish held in the acidic water had more gill chloride cells and mucous cells than those held in the limed water or the control river. The difference in chloride cell number was due to increased numbers of the cells on the primary lamellar epithelium; numbers of cells on the secondary lamellae did not increase with acid exposure. Male fish were found to have more chloride cells on their secondary lamellae than female fish. Chloride cells were larger and more nearly spherical in shape in the fish exposed to low pH water. The liming treatment was partially effective in preventing changes in gill histology. Changes in size, shape, and number of chloride cells probably represent a response of increasing ionic uptake to offset the losses of ions occurring during low pH stress.

Les branchies de saumon de l'Atlantique adulte, placés dans une rivière acide (rivière Westfield, comté de Queens en Nouvelle-Écosse; pH moyen de 4,8), ont été comparées à celles de poissons placés dans la même eau traitée à la chaux (pH moyen de 5,5) et à celles de poissons placés dans une rivière témoin avoisinante (rivière Medway; pH moyen de 5,4). D'après l'analyse morphométrique, les cellules acidophiles (dites à chlorure) et les cellules muqueuses étaient plus nombreuses chez les poissons placés dans l'eau acide que chez les poissons placés dans de l'eau chaulée ou dans la rivière témoin. La différence au niveau du nombre de cellules acidophiles était attribuable au nombre accru de cellules sur l'épithélium lamellaire primaire; le nombre de cellules sur les lamelles secondaires n'a pas augmenté avec une exposition à l'acide. Les lamelles secondaires des poissons mâles comptaient un plus grand nombre de cellules acidophiles que cells des poissons femelles. Les cellules acidophiles étaient plus grosses et beaucoup plus sphériques chez les poissons exposés à une eau à pH bas. Le traitement à la chaux a empêché partiellement les modifications histologiques des branchies. Les variations au niveau de la taille, de la forme et du nombre des cellules acidophiles représentent probablement une réaction à la fixation accrue des ions afin de compenser les pertes d'ions en présence d'une eau à pH faible.

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Adult Atlantic salmon (*Salmo salar*) enter rivers and migrate upstream in spring and summer to spawn in the fall before returning to the ocean. They are thus exposed to river waters for several months. It is generally accepted that acid precipitation is causing acidification of spawning rivers in Scandinavia (Jensen and Snekvik 1972; Muniz 1983). Some kills of adult Atlantic salmon have been reported (Leivestad et al 1976; Skogheim et al. 1984), but recruitment failure appears to be the main cause of declining populations (Leivestad et al. 1976; Rosseland et al. 1980). Salmon mortalities and population declines have been reported at pH 5.0–5.5 (Leivestad et al. 1976; Henriksen et al. 1984; Skogheim and Rosseland 1986). Farmer et al. (1980) and Watt et al. (1983) who surveyed the chemistry of rivers in southwestern Nova Scotia, and documented changes in the salmon fishery in these rivers, concluded that acid precipitation represented a serious threat to remaining salmon populations.

Neutralization of acidic waters by the addition of lime ($CaCO_3$) has been employed to restore and protect endangered fisheries in several rivers (Fraser and Britt 1982; Rosseland and Skogheim 1984). Liming water from an acid river has been shown to increase survival of salmon fry (Farmer et al. 1980), eggs, and smolt (Rosseland and Skogheim 1986).

Disruption of ion regulation is a principal effect of low pH exposure in fish (Wood and McDonald 1982; McDonald 1983). This ionoregulatory disturbance is reduced or eliminated when the pH of the water is raised by liming (Leivestad and Muniz 1976; Rosseland et al. 1984).

Gills are the primary organs of ion regulation in fish, and their function is strongly affected by acid water (McDonald 1983). Daye and Garside (1976, 1980) reported gill epithelial necrosis due to acute acid exposure, and Jagoe and Haines (1983) found low pH caused mucus accumulation and loss of surface microridges. Increased mucus production may be due

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TABLE 1. Chemistry of the Medway and Westfield rivers, Nova Scotia. Data from Lacroix (1985a, 1985b), Lacroix et al. (1985), and Lacroix and Kan (1986).

Parameter	Medway River	Westfield River
Total alkalinity (μeq/L)	20	10
Total aluminum (μg/L)	230	260
Exchangeable inorganic aluminum (μg/L)	20	30
Calcium (mg/L)	0.7	0.8
Chloride (mg/L)	4.3	3.5
Dissolved organic carbon (mg/L)	12.5	11.7
Magnesium (mg/L)	0.6	0.5
Potassium (mg/L)	0.3	0.3
Sodium (mg/L)	3.1	2.8
Sulfate (mg/L)	3.2	3.2
Specific conductance (μS/cm)	30	30

to mucous cell hyperplasia (Zuchelkowski et al. 1981; Haines et al. 1986) or hypertrophy (Daye and Garside 1976). Chloride cells appear to be responsible for ion uptake in fresh water (Laurent et al. 1985; Avella et al. 1987), and changes in their number and structure upon low pH exposure have been reported by a number of workers (Matey et al. 1981; Matey and Kharazova 1982; Leino and McCormick 1984; Matey 1984; Chevalier et al. 1985; Leino et al. 1987a, 1987b).

Given that neutralization of acid water diminishes or eliminates plasma ion losses caused by acid stress, it follows that changes in gill tissue associated with acid exposure should be prevented or reduced by liming. We compared chloride and mucous cell numbers, sizes, shapes, and distributions in gill tissues from adult salmon held in low pH, limed, and control waters.

Materials and Methods

Atlantic salmon (weight 600–1200 g) were exposed in groups of 40 to either Medway River water, untreated Westfield River water, or Westfield River water which had passed through limestone chips. Details of the source and handling of the fish, and the experimental treatments are given by Sangalang et al. (1990).

Daily measurements were made of the pH in each treatment (Sangalang et al. 1990). During the course of the study, mean pH in the Medway River was 5.4 (range 5.3–5.6), mean pH in the Westfield River was 4.8 (range 4.7–5.2), and the mean pH of the limed Westfield water was 5.5 (5.1–5.9). Water chemistry parameters of these rivers (Table 1) have been intensively studied by Lacroix (1985a, 1985b), Lacroix et al. (1985) and Lacroix and Kan (1986). The rivers are nearly identical in the ionic composition of their waters. Both rivers have fairly high total aluminum concentrations, but speciation experiments have demonstrated that virtually all of this is organically bound (Lacroix and Kan 1986), and thus non-toxic.

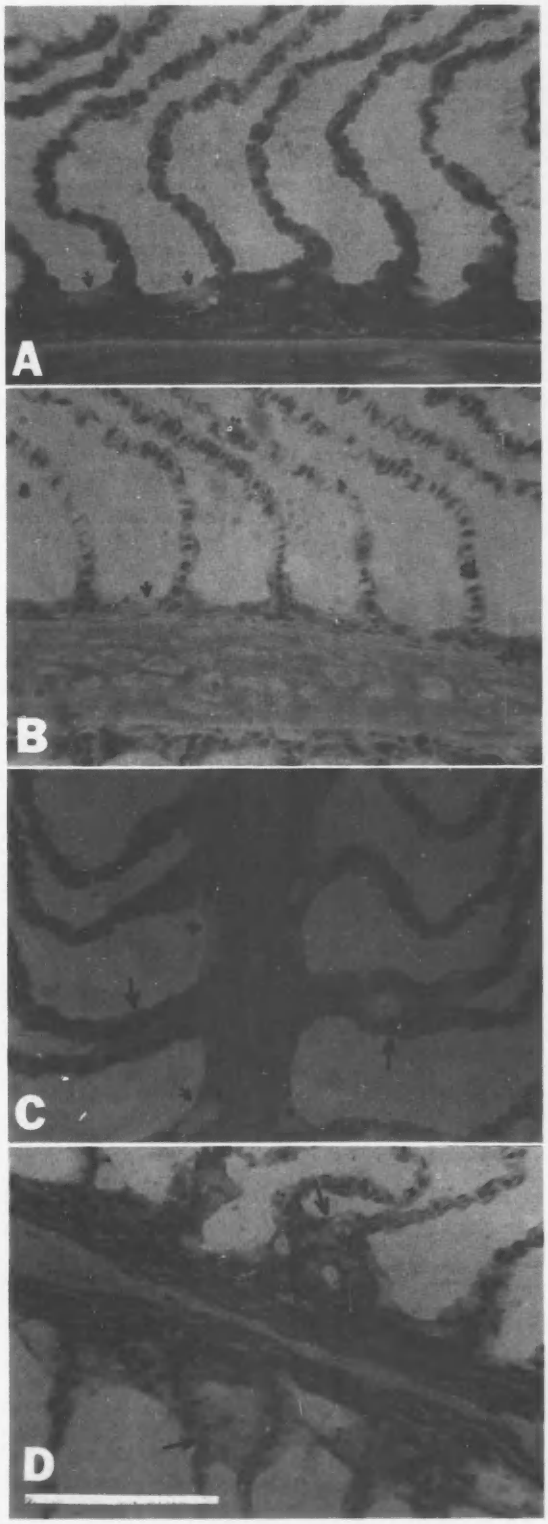
Dissolved oxygen and ammonia were not measured during the experiment, but there was no evidence of increased stress or mortality during warmer, low water periods. At low pH, virtually all ammonia is in the form of ammonium ion, which is nontoxic (Wuhrmann and Woker 1948), At 0–10°C, pH 5, less than 0.002% of the total ammonia present is in the toxic, unionized form (Messer et al. 1984). Thus, the total ammonia concentration in the tanks would have had to exceed 1000 mg/L to yield an ionized ammonia level of 0.02 mg/L, which is considered deleterious to freshwater fish (United States Environmental Protection Agency 1976; American Public Health Association 1985). Considering the total weight of the fish used, the volume of the tanks, and the flow rates (Sangalang et al. 1990), it is highly unlikely that this concentration was approached.

After approximately 3 mo of exposure to the three treatments (Medway, Westfield untreated, and Westfield limed), gill samples were collected on 9–10 December 1986. Fish were captured with dip nets and placed in an anaesthetic solution of MS-222 (tricaine methanesulfonate). Fish were processed sequentially in groups of two, so that all remained in the anaesthetic for the same period of time. When the fish became quiescent, the second gill arch on the right side was quickly dissected out, and placed in chilled fixative composed of 1% glutaraldehyde (Kodak) and 4% formaldehyde (from paraformaldehyde, Electron Microscopy Sciences) in 0.1 M phosphate buffer (pH 7.4) containing 10% sucrose (McDowell 1978). Sex was determined by dissection.

Gills were stored under refrigeration in this solution for several weeks. Several individual filaments were dissected free from the central portion (bend) of each gill arch, dehydrated to absolute ethanol through a graded ethanol series, rinsed in propylene oxide, and embedded in epoxy (a mixture of Epon and Araldite resins). We selected the longer member of each filament pair, thus ensuring that all filaments examined came from the same hemibranch. Filaments were sectioned longitudinally, parallel to the primary lamella and perpendicular to the secondary lamellae. This orientation has been used by other workers studying effects of acidification on gills (Leino and McCormick 1984; Chevalier et al. 1985; Leino et al. 1987a, 1987b; Evans et al. 1988) and was chosen to facilitate comparison. Sections (1 micron thick) were cut and mounted using the techniques described by Smith and Tyler (1984).

Slides were examined at 1000× using an oil immersion objective. Sections from the central areas of the filament were used for morphometric determinations. Cell counts, and cell profile area, perimeter, maximum diameter, and shape measurements were acquired directly from slides using a Zeiss Videoplan image analysis system. The system was calibrated using a stage micrometer (American Optical) before each period of use. Shape was defined as the ratio of the y axis to the x axis, where x was the maximum diameter of the cell, and y the length of a line from cell boundary to cell boundary which crossed the center of the x axis at an angle of 90°. Data were transferred to an IBM mainframe computer for analysis using the Statistical Analysis System (SAS Institute, Cary, North Carolina).

In previous studies of gills, nested analysis of variance has shown that most differences in numbers and sizes of chloride and mucous cells in populations were due to within-individual variation, rather than inter-individual differences (Haines et al. 1986; Jagoe 1988). While there was significant variation among individuals, this variation was relatively small, as has been reported in some tissues where strict limits are placed upon structural variation by functional requirements (Haines et al. 1986; Egginton 1988). In other words, much of the morphometric variation observed resulted from the particular filament selected, the angle at which the sections were cut, the exact location sampled within the filament, and the region of the slide chosen for examination, rather than differences between individuals. Therefore, an experimental design incorporating a large number of measurements per individual from a relatively small number of individuals was employed.



We randomly selected eight fish from each of the three experimental treatments for morphometric analysis. Five sequential slides, each containing ribbons of 10 to 20 sections, were prepared from each of two filaments from each individual. The first, third, and fifth slides from each filament were stained with 1% toluidine blue to visualize chloride cells. Chloride cells occurring on the primary lamellae were recorded separately from those occurring on the secondary lamellae. The proportion of chloride cells occurring on the primary lamellar epithelium was calculated according to the formula number in primary epithelium/(number in primary epithelium + number in secondary epithelium) for each interlamellar space (the region defined by two adjacent secondary lamellae, consisting of primary and secondary lamellar epithelia). Counts and measurements were made of chloride cells in 10 interlamellar spaces per section, 2 sections per slide, 3 slides per filament, and 2 filaments per individual.

The second and fourth slides from each filament were stained with periodic acid-Schiffs reagent (Humason 1967) to demonstrate mucous cells. Counts and measurements were made of mucous cells in a region defined by 10 adjoining secondary lamellae and their associated interlamellar spaces. The cells were counted and measured in two regions per slide, two slides per filament, and two filaments per individual.

Results

General Observations

In fish exposed to untreated, acidic Westfield River water, primary lamellar epithelia appeared somewhat thickened (Figure 1A). In many interlamellar spaces in these fish, several large chloride cells were found grouped together at the bases of the secondary lamellae, often completely covering the primary lamella. In fish held in limed Westfield water or in Medway water, the primary lamellar epithelia appeared relatively thin, with individual chloride cells (Fig. 1B). Adjacent secondary lamellae were sometimes partly fused (Fig. 1C). Several layers of chloride cells were observed in some specimens, and serial sections revealed that these formed a pore or pit lined with chloride cells (Fig. 1D). Counts were made of such abnormalities in slides from a few individuals held in untreated Westfield water. The percentage of secondary lamellae involved in the fusions ranged from nearly 25% to less than 1%. Based on these counts, we estimated that the fusions involved 2–5% of the interlamellar spaces in these fish. We saw no chloride cell proliferation or fusion of adjacent lamellae in any fish held in limed Westfield or Medway water.

Sampling Design

Cell count data for both mucous cells and chloride cells were analyzed using nested analysis of variance. Interindividual variance was smaller than intraindividual variance (Table 2). For chloride and mucous cells, differences between individuals

FIG. 1. Micrographs of gill tissue from Atlantic salmon held in the Medway and Westfield rivers. Scale bar = 100 micrometres. pl = primary lamella, sl = secondary lamella. Small arrows indicate representative chloride cells. Large arrows indicate lamellar fusions, and sites of chloride cell proliferation. A. Untreated Westfield River water; B. Medway River water, C. Untreated Westfield River water, D. Untreated Westfield River water.

TABLE 2. Nested analyses of variance of mucous cell and chloride cell counts in the gills of Atlantic salmon held in the Medway and Westfield rivers in 1986.

Level	df	Mean square	Variance comp.	(%)
Mucous cells				
Total	191	71.12	81.70	100
Treatment	2	1903.66	26.25	32.13
Individual	21	223.65	22.19	27.17
Filament	24	46.06	4.96	6.07
Slide	48	26.20	−1.04	0
Within Slides	96	28.29	28.29	34.63
Chloride cells				
Total	2879	3.86	4.02	100
Treatment	2	476.42	0.39	9.80
Individual	21	97.75	0.68	16.91
Filament	24	16.07	0.18	4.46
Slide	98	5.27	0.13	3.19
Section	143	2.75	0.01	0.31
Within Sections	2591	2.63	2.63	65.32

within treatments accounted for only about 17 and 27% of the total variance, respectively. However, variation at this level was significant for both cell types (mucous cells: $F = 4.86$, chloride cells: $F = 6.10$). Differences between filaments within individuals were also significant. Variation at the lowest sampling levels, between fields within slides or sections, accounted for the largest percentage of the total variance observed for each cell type. Similar results were obtained when a nested analysis of variance was performed on variables other than cell number. Interindividual differences within treatments accounted for 4.9% of the total variance of chloride cell area, and intraindividual variation accounted for 86.3% of the total. The present sampling design, which used large numbers of determinations from a small number of individuals, was therefore appropriate for this study.

Chloride Cell Numbers

Numbers of gill chloride cells were significantly greater in the fish held in untreated, acidic Westfield River water than in those held in the Medway River or in limed Westfield water (Table 3). Chloride cells were significantly fewer in fish held in the limed water than in those from the Medway River.

To investigate the differences among treatments in numbers of primary and secondary lamellar chloride cells, we examined the proportion of cells found on each type of epithelia. The percentages of chloride cells in the primary lamellar epithelium were 62 in fish held in the untreated Westfield water, 57 in limed Westfield water, and 48 in Medway River water. Analysis of variance on arcsin transformed proportions (Sokal and Rohlf 1969), demonstrated that all were significantly different ($F = 57.68$, $P<0.001$).

When numbers of chloride cells on primary and secondary lamellae were examined separately, it appeared that they responded differently to the treatments, producing the differences in proportion noted above. Primary lamellar chloride cell numbers were significantly different in all locations, and were highest in fish from the untreated Westfield water (Table 3). In contrast to the total chloride cell counts, however, primary lamellar chloride cell counts were higher in fish held in the limed Westfield water than in those from the Medway river. Secondary lamellar chloride cell counts were lowest in the fish from the limed Westfield water (Table 3); counts for fish held in untreated Westfield water were not significantly different from those for fish held in the Medway River.

Chloride cell numbers were associated with the sex of the fish: analysis of variance revealed that total numbers of chloride cells were significantly higher in males than in females ($F=45.21$, $P<0.001$). We analyzed the effects of the treatments separately on males and females, and found that fish of both sexes had significantly greater total chloride cell numbers in the untreated Westfield River water than in the limed Westfield River water or Medway River (Table 4). Primary lamellar chloride cell numbers were also elevated in both males and females in untreated Westfield water. Secondary lamellar chloride cell numbers were lowest in the limed Westfield water, and did not differ between the untreated Westfield and Medway samples in either sex.

Chloride cell numbers were not associated with fish size. Mean chloride cell numbers were not correlated with fish length (mean primary lamellar chloride cell number vs. total length, $r = 0.005$, $P>0.5$; mean secondary lamellar chloride cell number vs. total length, $r = 0.194$, $P>0.3$).

Sizes of Chloride Cells

As judged by cell profile area measurements, chloride cells were largest in fish held in untreated Westfield River water, of intermediate size in those held in untreated Westfield River water, and smallest in fish held in the Medway River (Table 5). As in the counts of chloride cells, we saw somewhat different relationships between the treatments when we examined primary and secondary lamellar chloride cells separately (Table 5). Primary lamellar chloride cells were larger in fish from untreated and limed Westfield River water than in fish held in the Medway River. There was no difference in size of the primary lamellar chloride cells between fish from the treated and untreated Westfield River water. Secondary lamellar chloride cells were smallest in fish held in the Medway River, and largest in fish held in untreated Westfield water. Mean size of the cells differed significantly among all three treatments.

Sizes of chloride cells were not related to size or sex of the fish. The sizes of primary and secondary lamellar chloride cells were the same in males and females.

TABLE 3. Effects of low pH and liming on primary lamellar, secondary lamellar, and total chloride cell numbers in Atlantic salmon. Data are given as mean number of chloride cells per interlamellar space ± SE. Means with the same superscript are not significantly different. $N = 960$ for each.

	Treatment				
Chloride cells	Medway	Westfield	Limed Westfield	F	P
Total	$3.33^a \pm 0.060$	$4.43^b \pm 0.070$	$3.12^c \pm 0.050$	134.94	0.001
Primary lamellar	$1.40^a \pm 0.030$	$2.56^b \pm 0.042$	$1.66^c \pm 0.032$	303.88	0.001
Secondary lamellar	$1.92^a \pm 0.051$	$1.87^a \pm 0.055$	$1.47^b \pm 0.041$	25.14	0.001

TABLE 4. Effects of low pH and liming on primary lamellar, secondary lamellar, and total chloride cell numbers in male and female Atlantic salmon. Data are given as mean chloride cell count per interlamellar space ± SE. *N* is number of individuals per treatment, 120 interlamellar spaces per individual. Means with the same superscript are not significantly different.

Variable	Treatment			*F*	*P*
	Medway	Westfield	Limed Westfield		
Females					
N	4	4	6		
Total	$3.04^{a} \pm 0.084$	$4.36^{b} \pm 0.085$	$3.05^{a} \pm 0.056$	100.77	0.001
Primary	$1.33^{a} \pm 0.041$	$2.73^{b} \pm 0.058$	$1.74^{c} \pm 0.038$	217.72	0.001
Secondary	$1.71^{a} \pm 0.070$	$1.63^{a} \pm 0.067$	$1.30^{b} \pm 0.042$	15.60	0.001
Males					
N	4	4	2		
Total	$3.61^{a} \pm 0.085$	$4.51^{b} \pm 0.110$	$3.35^{c} \pm 0.056$	33.61	0.001
Primary	$1.47^{a} \pm 0.042$	$2.40^{b} \pm 0.059$	$1.39^{c} \pm 0.055$	112.49	0.001
Secondary	$2.14^{a} \pm 0.074$	$2.11^{a} \pm 0.087$	$1.96^{b} \pm 0.099$	0.8	ns

TABLE 5. Effects of low pH and liming on primary lamellar, secondary lamellar, and total chloride cell areas. Data are presented as mean cell profile area in square microns ± SE with associated (*N*). Means with the same superscript are not significantly different.

Chloride cells	Treatment			*F*	*P*
	Medway	Westfield	Limed Westfield		
Total	$71.14^{a} \pm 0.371$ (3188)	$89.73^{b} \pm 0.535$ (4229)	$85.16^{c} \pm 0.566$ (2994)	362.21	0.001
Primary lamellar	$77.23^{a} \pm 0.601$ (1348)	$93.61^{b} \pm 0.683$ (2426)	$95.11^{b} \pm 0.802$ (1581)	156.36	0.001
Secondary lamellar	$66.67^{a} \pm 0.442$ (1840)	$84.50^{b} \pm 0.838$ (1803)	$74.03^{c} \pm 0.683$ (1413)	190.25	0.001

TABLE 6. Effects of low pH and liming on primary lamellar, secondary lamellar, and total chloride cell shapes. The shape factor can range from approaching zero (flat) to one (circular). Data are given as mean shape factor ± SE with associated (*N*). Means with the same superscript are not significantly different.

Chloride cells	Treatment			*F*	*P*
	Medway	Westfield	Limed Westfield		
Total	$0.46^{a} \pm 0.003$ (3188)	$0.54^{b} \pm 0.003$ (4229)	$0.49^{c} \pm 0.003$ (2994)	238.88	0.001
Primary lamellar	$0.53^{a} \pm 0.005$ (1348)	$0.55^{b} \pm 0.003$ (2426)	$0.52^{a} \pm 0.004$ (1581)	19.54	0.001
Secondary lamellar	$0.41^{a} \pm 0.003$ (1840)	$0.53^{b} \pm 0.004$ (1803)	$0.44^{c} \pm 0.004$ (1413)	285.27	0.001

Shapes of Chloride Cells

Measurements of cell size other than area (e.g. cell diameter and cell perimeter) should yield results similar to those above if the cells are the same shape. However, this was not always the case, suggesting that there might be differences in chloride cell shape among the treatment groups. For example, chloride cell perimeters (primary and secondary lamellar chloride cells pooled) were largest in fish exposed to the limed Westfield River water, intermediate in the untreated Westfield River fish, and smallest in the Medway River fish ($F = 37.65$, $P<0.001$), whereas the areas for these cells were greatest in fish from the untreated Westfield River water. Similarly, the diameters of secondary lamellar chloride cells were smaller in fish held in untreated Westfield River water than in fish exposed to limed Westfield or Medway waters ($F = 17.59$, $P<0.001$). Yet again, areas of these cells were greatest in the untreated Westfield sample. Such differences are best explained by differences in shape of chloride cells among the treatments.

For parametric analysis of the shape factor (Y diameter/X diameter), the ratio was transformed using the arcsin transformation (Sokal and Rohlf 1969). Shapes of chloride cells were significantly different among treatments (Table 6). The cells were most spherical in fish held in the untreated Westfield River water, and flattest in fish held in the Medway River. The results were the same when the primary and secondary lamellar chloride cells were analyzed separately. Both primary and secondary lamellar chloride cells were significantly more spherical in fish held in the most acidic water (untreated Westfield River,

TABLE 7. Comparisons between chloride cells occurring in primary lamellar epithelia and chloride cells occurring in secondary lamellar epithelia in Atlantic salmon. Comparisons are made separately for each location. Data are given as means ± SE with associated (*N*). Area is microns^2.

Variable	Treatment		
	Medway	Westfield	Limed Westfield
Area			
Primary	77.23 ± 0.601	93.61 ± 0.683	95.11 ± 0.802
(*N*)	(1348)	(2426)	(1581)
Secondary	66.67 ± 0.442	84.50 ± 0.838	74.04 ± 0.683
(*N*)	(1840)	(1803)	(1413)
F	210.13	72.18	390.65
(*P*)	(<0.001)	(<0.001)	(<0.001)
Number per interlamellar space			
Primary	1.40 ± 0.033	2.56 ± 0.042	1.66 ± 0.032
(*N*)	(960)	(960)	(960)
Secondary	1.92 ± 0.051	1.87 ± 0.055	1.47 ± 0.041
(*N*)	(960)	(960)	(960)
F	76.71	99.10	12.16
(*P*)	(<0.001)	(<0.001)	(<0.001)
Shape			
Primary	0.53 ± 0.005	0.55 ± 0.003	0.52 ± 0.004
(*N*)	(1348)	(2426)	(1581)
Secondary	0.41 ± 0.003	0.53 ± 0.004	0.45 ± 0.004
(*N*)	(1840)	(1803)	(1413)
F	466.65	13.84	163.54
(*P*)	(<0.001)	(<0.001)	(<0.001)

Table 6). The shape of chloride cells did not differ between sexes.

Comparisons Between Primary and Secondary Lamellar Epithelia

Chloride cells on primary and secondary lamellar epithelia differed significantly in area, number and shape (Table 7). Each of the three groups of fish were analyzed separately to remove effects of treatment. In each group, primary lamellar chloride cells were significantly larger than secondary lamellar ones. Shape ratios of primary lamellar chloride cells were greater than those of cells on secondary lamellae, indicating that chloride cells on the primary lamellae were more nearly spherical. In the control (Medway) fish, more chloride cells were on secondary lamellae than on primary lamellae, whereas in the other two treatments this distribution was reversed.

Size, Number, and Shape of Mucous Cells

The profile area of mucous cells differed significantly among the treatments (Table 8). Mucous cells in fish held in the Medway River were largest, and those in fish held in the limed Westfield water the smallest. There were no differences in mucous cell shape among the treatments. Mucous cells were more numerous in gills of fish held in untreated Westfield River water, and least numerous in fish held in the Medway River (Table 8). There were no differences between sexes in number, profile area, or shape of mucous cells.

Discussion

Fraser and Britt (1982) concluded that allowing stream water to flow over and through crushed limestone could raise stream pH, but that it was not a particularly effective mitigation technique. In the present study, passage of Westfield River water over a bed of crushed limestone raised the pH of the water 0.5 to 1 pH unit, so that the mean pH in the limed treatment was the same as that in the Medway River (Sangalang et al. 1990). However, pH varied over a wider range and at more frequent intervals in the limed Westfield water as compared to the Medway River. This treatment was also not completely effective in eliminating the effects of exposure to the acidic Westfield River water in the fish, because there were differences between gills of control fish and those held in the limed treatment. These differences probably resulted because fish held in the limed Westfield water were exposed to lower pH than their counterparts in the Medway River, as well as experiencing pH fluctuations of greater magnitude. Our observations of differences in gill structure between fish in constant pH and fluctuating pH environments, despite similar mean pH values, is consistent with that of Hobe and McMahon (1988), who showed that exposure to a fluctuating pH regime caused a different pattern of acid-base and ionoregulatory stress than exposure to constant low pH in white sucker *Catostomus commersoni*.

Increased numbers of chloride cells with acid exposure were reported in fathead minnows *Pimephales promelas* by Leino and McCormick (1984), brook trout *Salvelinus fontinalis* by Chevalier et al. (1985) and Tietge et al. (1988), and pearl dace *Semotilus margarita* by Leino et al. (1987b). Leino and McCormick (1984) and Leino et al. (1987b) found that secondary lamellar chloride cells proliferate upon exposure to low pH. They also found increased percentages of chloride cells in secondary lamellar epithelium as compared to primary lamellar epithelium with acid exposure, presumably due to increased numbers of secondary lamellar chloride cells. In our study, the numbers of secondary lamellar chloride cells were not greater in the fish held in the lower pH river, but primary lamellar chloride cell numbers were significantly increased. Also, the percentages of the chloride cells on the secondary lamellar epithelium were slightly but significantly decreased in fish exposed to untreated acid river water. This resulted from increased numbers of chloride cells on the primary lamellae, and not decreased numbers on the secondary lamellae. In the present study, the percentage of chloride cells in secondary lamellar epithelium in the controls was greater than that reported for either fathead minnows (Leino and McCormick 1984) or pearl dace (Leino et al. 1987b).

Increases in numbers of secondary lamellar chloride cells have also been reported when fish have been exposed to waters of low ionic strength (Laurent and Dunel 1980; Laurent et al. 1985; Avella et al. 1987), and upon exposure to a variety of toxicants (Mallat 1985). Recently, Leino et al. (1987a) reported that secondary lamellar chloride cells in fathead minnows proliferated in soft water, but that acidification of the soft water caused a decrease in their number. Primary lamellar chloride cell numbers were not affected by their treatment. They attempted to reconcile these results with earlier findings of increased secondary lamellar chloride cell numbers with acidification by noting that the earlier studies had been done in considerably harder water.

To better understand the observed effects, it is also necessary to understand the dynamic processes controlling numbers and distribution of the cells. Cell renewal in gills has been studied by Conte and Lin (1967), MacKinnon and Enesco (1980), and Zenker et al. (1987) using tritiated thymidine, which becomes incorporated into cell nuclei during DNA replication. Using

TABLE 8. Effects of low pH and liming on mucous cell size, number, and shape in Atlantic salmon. Data are given as means ± SE with associated (*N*). Area is microns^2, and number is per 10 interlamellar spaces.

	Treatment				
Variable	Medway	Westfield	Limed Westfield	*F*	*P*
Area	$40.44^a \pm 0.85$ (487)	$35.26^b \pm 0.45$ (1185)	$33.19^c \pm 0.47$ (847)	33.93	0.001
Number	$7.61^a \pm 0.60$ (960)	$18.52^b \pm 1.02$ (960)	$13.22^c \pm 1.02$ (960)	36.80	0.001
Shape	0.60 ± 0.007 (487)	0.59 ± 0.004 (1185)	0.60 ± 0.005 (847)	0.75	ns

pulse injections of the radioactive material and autoradiography of tissues at selected intervals after administration, they were able to identify sites of active cell division, and track migration of the cells away from the areas of division and differentiation. The results of these studies support the idea that gill epithelial cells, including chloride cells, arise in primary lamellar epithelium and then migrate out onto the secondary lamellae. There was little cell division on the secondary lamellae. Thus, the two epithelia — either structurally or functionally — are not completely distinct (Laurent et al. 1985).

Increased thickness of the primary lamellar epithelium after exposure to low pH observed in this study and others (Chevalier et al. 1985; Leino et al. 1987a, 1987b) results from proliferation of undifferentiated stem cells and the maturation of chloride cells. This proliferation would fit with increased production and differentiation of chloride cells, resulting in the increased numbers of primary lamellar chloride cells observed in our study. Some cells would migrate out onto the secondary lamellae and differentiate. If the rate of loss of chloride cells from the secondary lamellae was increased by acid damage to the cells, as suggested by Leino et al. (1987a), one would see increased numbers of primary lamellar chloride cells and unchanged numbers of secondary lamellar chloride cells as reported here. Alternatively, there could be a decrease in amount of migration of the cells onto the secondary lamellae, which would also produce this distribution.

Fish held in limed Westfield water had a lower total chloride cell number than controls, and their distribution was somewhat different. There were slightly more primary lamellar chloride cells and fewer secondary lamellar chloride cells. As previously noted, secondary lamellar chloride cells proliferate in fish exposed to very soft or distilled water, and thus appear to be involved with increased ionic uptake (Laurent et al. 1985). The decrease in numbers of these cells with liming therefore suggests that the fish have a lower requirement for uptake of ions. This is not surprising; presumably the fish were experiencing less ionoregulatory stress since the liming treatment raised the water pH and ionic strength. Secondary lamellar chloride cells have also been implicated in calcium transport (Payan et al. 1981), so a lesser number of them might be related to a decreased need for calcium uptake in higher pH, higher calcium water.

Within each treatment group, secondary lamellar chloride cells were consistently smaller and flatter than primary lamellar chloride cells. This may be related to biomechanical considerations. Primary lamellar chloride cells sit deeply in the surrounding epithelium. If secondary lamellar chloride cells were larger and more rounded, they would project out from the adjacent thin secondary lamellar pavement cells, and could interfere with water flow across the gills. Exposure to dilute waters causes secondary lamellar chloride cells to become more spherical in shape (Laurent et al. 1985; Avella et al. 1987), but the results here suggest that they may tend to remain smaller and flatter than their primary lamellar counterparts.

To date, few observations of changes in chloride cell size due to exposure to acid have been reported. Chevalier et al. (1985) reported chloride cell hypertrophy in fish from acidified lakes (but they did not quantify it), and Matey and Kharazova (1982) reported it in the carp *Carassius carassias* exposed to pH 4.5 for 1 wk. There are apparently no previous quantitative descriptions in the literature of altered chloride cell shapes with low pH exposure, although Avella et al. (1987) recently showed that exposure to very high calcium (400 mg/L) or low calcium water (2.4 mg/L) produced a greater proportion of spherical chloride cells than exposure to water containing 64 mg/L calcium. Calcium concentrations in both the Medway and Westfield rivers are less than 1 mg/L (Lacroix 1985a, 1985b; Lacroix et al. 1985; Lacroix and Kan 1986), and based on the pH elevation observed in the liming treatment, it is not likely that calcium concentration there exceeded 2 mg/L. Thus, all treatments in our experiment were very low in calcium, and calcium concentrations did not vary sufficiently to explain the changes in cell shape.

Chloride cell hypertrophy has been reported as a response to toxicants such as copper (Baker 1969) and nitrite (Gaino et al. 1984), as well as upon acclimation to seawater (Laurent and Dunel 1980). It is generally accepted by these and other workers that increased size reflects increased ionic transporting activity. Likewise, Laurent et al. (1985) argued that alteration in chloride cell shape (bulging) is a method of increasing the surface area of the cell exposed to the water. Since apical ion exchange mechanisms are involved in ion uptake (Evans 1975), this would increase the area available for transport sites, and therefore presumably reflects increased transport activity (Avella et al. 1987). A number of studies have shown that influxes of sodium and chloride are inhibited by low pH in salmonid fishes, and these influxes remain inhibited, or partially recover over longer exposure (McDonald et al. 1983; Booth et al. 1988; Wood et al. 1988). Wood et al. (1988) suggested that chloride cell proliferation may be a response to the dual problems of inhibited ionic uptake and increased ionic efflux. Thus, all of the alterations in chloride cell size, shape, and number with acid exposure that we report here are consistent with the fish attempting to increase ionic uptake in response to low pH-induced ionic losses.

Because the Westfield River fish were held in tanks receiving treated or untreated river water, while the Medway River fish were held in a floating net cage, the criticism could be made

that differences in ammonia or dissolved oxygen concentrations between the treatments were responsible for some of the alterations in gill morphology. We did not measure dissolved oxygen or ammonia, but as discussed above, it is unlikely that either reached stressful levels. Lifting and separation of the epithelial layer, breakdown of the pillar cell system, and development of secondary lamellar aneurysms with ammonia exposure were reported by Bullock (1972) and Smart (1976). Neither observed hyperplasia of undifferentiated epithelial cells in either acute or chronic exposure, as was reported by Burrows (1964). There is little literature on any effects of low dissolved oxygen on gill structure, but Larmoyeux and Piper (1973) found edematous fused lamellae and lamellar aneurysms in rainbow trout exposed to low dissolved oxygen and elevated ammonia. Mallat (1985) cited 10 studies of effects of ammonia on gill structure, and none of them noted chloride cell or mucous cell proliferation. The changes in gill structure we observed were different than those associated with elevated ammonia and low dissolved oxygen concentrations, so we conclude that any differences in dissolved oxygen or ammonia levels among the treatments did not affect the gill structures we measured.

The sexual dimorphism in chloride cell number noted here is without precedent in the literature. Leino and McCormick (1984) reported that there was no difference between males and females, and other workers seem not to have addressed this question. Because the numbers of chloride cells increased in both sexes upon acid exposure, the difference in actual number between males and females did not affect our conclusions. However, testing for such effects in future studies is essential, lest incorrect conclusions be drawn as an artifact of unequal sex ratios.

Zuchelkowski et al. (1986) showed sexual dimorphism in the numbers and sizes of skin mucous cells of brown bullheads (*Ictalurus nebulosus*) after acid exposure. We found no difference in numbers, sizes, or shapes of mucous cells between sexes, but the cells were more numerous in fish held in acidic water. This result is consistent with those of Zuchelkowski et al. (1981), who showed that mucous cell hyperplasia occurred in the skin of brown bullheads exposed to low pH; and of Haines et al. (1986) who found increased numbers of mucous cells in the gills of white sucker (*Catostomus commersoni*) from an acid lake. Zuchelkowski et al. (1981) and Haines et al. (1986) found no difference in mucous cell size associated with lower pH. Daye and Garside (1976) reported mucous cell hypertrophy in fingerling brook trout exposed to acid water. We found that mucous cells in the fish held in the acid water were smaller than those from the controls. The reason for this is unclear, but it seems possible that the cells in fish exposed to low pH might be cycling faster; that is, filling with somewhat less mucous before discharging. Given that there are considerably more mucous cells present at low pH, this would still allow production of a greater amount of mucous that could be renewed more rapidly.

Mucus production is a generalized response to a variety of stresses (Zuchelkowski et al. 1981; Mallat 1985), and it has been suggested that altered glycoprotein secretion is an adaptive response to osmotic stress (Solanki and Banjamin 1982; Zuchelkowski et al. 1985). Fish mucus contains calcium binding proteins (Flik et al. 1984) in a highly negative, polyanionic matrix that may be involved in facilitating ion uptake by concentrating ions near the epithelial surface (Marshall 1978). These functions are still the subject of debate, with some evidence indicating that mucus plays a minor role in sequestering calcium (Part and Lock 1983) and monovalent cations (Stith 1984). Gill mucus production is clearly increased in acid stressed fish (Daye and Garside 1976; Jagoe and Haines 1983) and the increased thickness of the mucous layer has been implicated in decreased capacity for gas exchange, leading to anoxia (Westfall 1945; Ultsch and Gros 1979).

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LETTERS AND COMMENTS/ LETTRES ET COMMENTAIRES

Comment on "Larval Fish Age, Growth, and Body Shrinkage: Information Available from Otoliths"

In a recent paper appearing in the *Journal*, Radtke (1989) reported on the age, growth, and body shrinkage of larvae of Atlantic cod (*Gadus morhua*). We have many concerns with this work which we describe below, grouped by category.

Methodological Concerns

We believe that experimental protocol is incompletely described in the 1989 paper. For example, fig. 5A presents a scatterplot of increment number versus age which demonstrates that many of the larvae <12 d old did not produce increments on a daily basis. Dr. Radtke attributed the discrepancy to the presence of moribund fish, which were earlier implied to be nonfeeding. The experimental conditions which the larvae were exposed to were not given, except to make reference to cultures 1 and 2 in the figure caption. In a later statement, Dr. Radtke reported that 10-d-old larvae in an intentionally starved treatment were healthy and characterized by a complete sequence of daily increments, albeit faint, a point reiterated in the Discussion. Given such contradictory statements, it remains unclear whether starved fish always produce increments, and if so, did Dr. Radtke overlook such poorly defined structures in the examination of otoliths used in the preparation of fig. 5A.

Poor documentation may have been responsible for the apparent discrepancy between the stated and graphically presented growth rates of larvae held under three different temperatures. The minimal difference (≈10%) among growth rates reported in the text was not consistent with the 29% difference in length at age evident in fig. 7A, although peak instantaneous growth rates may have indeed differed by less than 10%. If Dr. Radtke had intended the reference to minimal growth rate differences to apply only to peak instantaneous rates, it was not stated as such, nor was it clear why he would want to do so.

Dr. Radtke's use of Steffensen's (1980) methodology for the preparation of large sagittae may have resulted in unfortunate errors. Steffensen himself expressed concern that his technique may have obliterated a large sequence of larval growth increments, a concern which has since been substantiated in two independent studies of cod otoliths (Bolz and Lough 1983; Campana and Hurley 1989). Thus, Dr. Radtke's citation of Steffensen's (1980) results in support of his own growth rate estimates may be similarly flawed.

Mistaken Inferences and Unfounded Assumptions

Dr. Radtke distinguished three major landmarks during the ontogeny of cod otoliths: a check or mark at hatching, one associated with yolk-sac absorption, and another associated with metamorphosis. However, no example of a metamorphic check is provided for readers' scrutiny. Furthermore, he misinterprets the early life history of Atlantic cod by apparently using the terms "metamorphosis" and "settling" synonymously. Evidence of this is provided on p. 1888, where he notes that "... the fish reaches metamorphosis and begins a benthic lifestyle." In the Northwest Atlantic, metamorphosis of larval cod occurs during the pelagic phase at a size of 11–12 mm, and post-metamorphic fish remain largely in the pelagic zone for several months, typically recruiting to the demersal population in July (Lough et al. 1989). This misunderstanding affects interpretation of others' work, as indicated by his point of contrasting how in his laboratory-based study, he found "settling checks" whereas Bolz and Lough (1983) did not. Such differences are not surprising, as the latter authors examined presettling fish caught in midwater gear only. Dr. Radtke goes on to caution readers that incorrectly assigning an event to a mark on the otolith may be responsible for poor correspondence between observed and expected increment counts. Yet, he himself appears guilty of such an error by providing no examples of settling/metamorphic checks, and no description of how those and other checks were related to apparently important life history events.

Validity of Inferences Drawn from the Fish Length – Otolith Diameter Relationship

Dr. Radtke states that the relationship between live fish length and otolith length can be used to adjust for shrinkage in preserved samples. Such a statement explicitly assumes that there are no sources of systematic variation in the fish length – otolith length relationship. However, a survey of the literature indicates that systematic variations in the fish length – otolith dimension relationships are the rule, rather than the exception. We were able to find 15 published reports (Templeman and Squires 1956; Krivobok and Shatunovskiy 1976; Taubert and Coble 1977; Marshall and Parker 1982; McGurk 1984; Miller and Storck 1984; Boehlert 1985; Neilson et al. 1985; Penney and Evans 1985; Rice et al. 1985; Post and Prankevicius 1987; West and Larkin 1987; Mosegaard et al. 1988; Reznick et al. 1989; Secor and Dean 1989) citing statistically significant among-sample differences in the fish–otolith relationship. All of the above studies noted that slow-growing fish produced larger otoliths than faster-growing fish of the same size. The median among-sample difference in mean fish length at a given otolith diameter was 20%, a value which is comparable with the shrinkage which might be expected upon death and preservation. In contrast, we could not find any papers which reported similar fish length – otolith dimension regressions among samples characterized by different growth rates. Dr. Radtke did not present any data, figures, analyses, or results of analyses to support his position that the fish length – otolith dimension relationship was invariant. Indeed, while he cited Templeman and Squires (1956) as support for the statement that otolith size can provide a reliable estimate of fish length, Templeman and Squires actually emphasized the opposite. Thus, Dr. Radtke appears to have ignored a major source of error in the interpretation of fish length – otolith dimension data, and

erred in concluding that otolith size can provide a reliable estimate of fish length, independent of temperature.

Dr. Radtke's observations can be readily interpreted in terms of the response of the fish length – otolith dimension relationship to growth rate variations. For instance, departures from the postulated linear fish length – otolith diameter relationship in fig. 8B are probably due to changes in growth rate through the course of the experiment. A similar explanation appears likely for the three, apparently artifactual "growth stanzas" of fig. 8, none of which are evident in independent data (Bolz and Lough 1983; Campana 1989). While Dr. Radtke attributes the associated inflection points to the formation of accessory primordia, the latter do not form in cod larvae <10 mm in length (Bergstad 1984; S. E. Campana, unpublished). Failure to compensate for growth effects on the fish length – otolith diameter relationship could render Dr. Radtke's proposed shrinkage correction less reliable than the uncorrected lengths; the adjusted lengths for slow-growth samples would be artificially inflated above those of fast-growth samples.

As a test of the validity of Dr. Radtke's premise that the fish length – otolith diameter relationship in cod is stable and can be used to correct for preservation shrinkage, we applied his shrinkage equation to two independent cod larvae data sets. The live standard lengths reported by Bolz and Lough (1983) (corrected for shrinkage through use of Theilacker's (1980) algorithm) exceeded those of Radtke (1989) at equivalent otolith diameters by up to 26%. The discrepancy was even larger when Bolz and Lough's (1983) preserved lengths were compared with Radtke's (1989) preserved lengths. Since standard length is a shorter measure than the total length used by Radtke, the actual discrepancy between the two studies is greater yet. Similar results were obtained when Radtke's live fish length – otolith diameter relationship was compared with shrinkage-uncorrected data of Campana (1989): lengths in the former exceed those of the latter by up to 20%. When corrected for shrinkage through use of Radtke's equation, the reverse occurred: Campana's (1989) lengths exceeded those of Radtke (1989) by up to 20%. Since the interstudy differences in the fish length – otolith diameter relationship could not be reconciled under any plausible shrinkage scenario, it is clear that factors other than shrinkage are capable of introducing comparable levels of variation into the fish length – otolith diameter relationship.

Miscellaneous Concerns

Dr. Radtke introduces some new terminology without rigourous definition. Examples include "microincrements, settling checks, core checks." We object to the use of such terms when more accepted synonyms exist. Unrestricted growth of such jargon as those mentioned leads to lack of clarity in Radtke's paper and confusion in the subsequent literature. As a result of his prolific and well-recognized contributions to this field, we believe Dr. Radtke's work will be intensively studied by the growing number of workers in this field. As a consequence, he has an added responsibility to maintain consistency in using accepted terminology.

We would have appreciated more contrast between Dr. Radtke's findings and those of other workers who have examined the otolith microstructure of larval cod. In particular, Bergstad (1984), Dale (1984), and Campana and Hurley (1989) were available prior to the acceptance of Dr. Radtke's paper.

In the Materials and Methods and Acknowledgements sections, the author notes that the material for this study was obtained from experiments performed at the Flødevigen Biological Station, Norway, with financial support from the National Science Foundation. At least in part, this does not appear correct. Figure 2 of Radtke's (1989) paper, showing a sagittal otolith from a 3-d-old cod larva, has also appeared in a closely related study reported in Radtke and Waiwood (1980); this described work Dr. Radtke completed at the Biological Station, St. Andrews, that was supported by a Visiting Fellowship from the National Research Council of Canada and the Department of Fisheries and Oceans. Subsequently, the same well-travelled photomicrograph appears as fig. 4 in Radtke (1984), although the source is correctly acknowledged. Figure 6 of Radtke (1984), a scanning electron micrograph of an otolith from 7-d-old cod larva, also makes an encore appearance in the 1989 paper, without reference to the previous publication. Overall, we are not convinced that Radtke (1989) adheres strictly to the *Journal's* requirements for original information either in terms of the data pertaining to cod otolith microstructure or their interpretation — John D. Neilson, *Marine Fish Division, Biological Station, St. Andrews, N.B. E0G 2X0 Canada,* and Steven E. Campana, *Marine Fish Division, Bedford Institute of Oceanography, Dartmouth, N.S. B2Y 4A2 Canada.*
(JA507)

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Information Storage Capacity of Otoliths: Response to Neilson and Campana

My paper was condensed to almost half its original length in response to reviewers' comments. As a consequence, the methodology was shortened and the details requested by Neilson and Campana were deleted. Physical logistics of the rearing experiment were accomplished through the use of a filtered (flow-through) water system which is described in Fig. 1. Larvae were reared in 70-L cylinders emersed in a 100-L constant-temperature bath maintained at 6, 8, and 10°C for each respected rearing condition; sand-filtered water flowed through four cylinders for each temperature regime. Temperatures were maintained through the use of mixing ambient temperature seawater with heated seawater and monitored continuously.

In the results from the aging experiments, Neilson and Campana question the validity of interpretations which demonstrate that moribund larvae (i.e. larvae which I judged would almost certainly die, or were not adequately formed) did not produce daily increments. Actively swimming larvae that were judged to be "normal" had a daily increment count of a one to one relationship. "Normal" is an interpretation on my part that results from experience in rearing cod larvae and literature interpretations (Jones and Brothers 1987). It should be noted that many fish spawn a large number of eggs and most larvae never reach adulthood (Houde 1987). Many of these mortalities may be due to inefficiencies in development. (Hjort 1914; Blaxter 1974). While my experiments do not prove conclusively that increment formation may cease in response to ontogeny malfunctions, they do suggest it. Examination of all larvae without picking out the healthy ones can give a definition bias in the results of age versus increment count (Radtke 1989, fig. 5). This discrepancy in increment formation disappeared

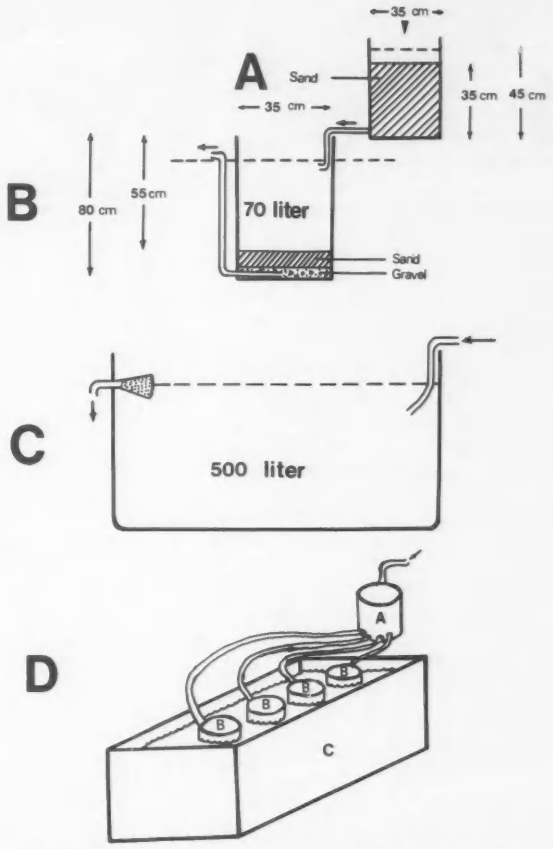

FIG. 1. Rearing apparatus for laboratory studies of cod (*Gadus morhua*). A, sand filter; B, incubation cylinder; C, water bath for temperature control; D, assembled apparatus.

after a major mortality event that corresponded to the time of the first feeding.

The time dimensions of a number of growth characteristics can be defined in considerable detail using otolith data. Opportunistic (pioneering) species have intrinsically high rates of population increase, high rates of ontogenetic growth, and high dispersal capabilities. Opportunists generally have short life spans and reproduce at early ages; mortality is usually high in these species. Species adapted to more mature or equilibrium situations usually have slower rates of population change and ontogenetic growth; reproduction is closely balanced by mortality. Information about such characteristics can be deduced from the record of growth processes left in otoliths.

In a separate experiment, actively moving and what were judged to be normally developed larvae were kept by themselves and starved at a temperature of 6°C. The fish were then examined when they were 10 d old. This was not a major part of the experiment per se, but was accomplished to examine what effects starvation would have on increment formation in fish that were able to feed. These fish were still in a healthy condition and were able to feed even after 10 d of starvation. I agree with Neilson and Campana that it remains unclear whether starved fish always produce increments or not, although this has been previously suggested by Jones and

Brothers (1987). My experiments suggest that moribund or maldeveloped fish do not produce daily increments, while active fish do. Discrepancies found in the literature (i.e. Geffen 1982) may be due to a nonseparation of normal and nonnormal fish. Thus, culture experiments may be biased. Additional experiments to examine the effects of starvation would clarify these data. I did not overlook incremental structures in the examinations of otoliths used in the preparation for Fig. 5A (Radtke 1989). And in fact I used scanning electron microscopy (SEM) to look for increments in the larvae that I judged to be morbid. Undoubtedly, further SEM studies and documentation of starvation may demonstrate increased differences.

The discrepancies stated between growth rates of the three larval groups and the differences in the length at age are not fully comparable relationships. Growth rates are rate per day functions, while length at age can refer to other criteria. The rate itself is the increase in length over time and, in fact, the growth rates in correspondence to length over time differed by less than 10%, or around 10%. I believe that the reference to the problem in discrepancy is not a crucial problem; and if Neilson and Campana would like to look at the data themselves I would be happy to provide such enumeration for them.

The complaint on the use of Steffensen's (1980) methodology is a distortion of my methodology: the reference to Steffensen was used solely to describe the plane surface for which the SEM sections were prepared (Radtke 1989, p. 1885). Before the SEM methodology was established, the otolith was sectioned in different planes as described by Radtke (1987) to determine the optimum plane for increment enumeration. Consequently, it is felt that no increments were lost and the plane in which the increments were counted was concluded to be the ideal plane. In healthy growing fish, it is believed that the present techniques did not obliterate any increments and the utilization of Radtke (1987) techniques were the best techniques to substantiate daily increments by SEM. Nevertheless, no significant deviation from daily increment deposition was found in laboratory larvae at any stage, especially in those which fed actively. Rates of increment formation, otolith growth, and body growth both increased from a low level during the first 20 d of life to a higher level during the rest of the larval period. Daily increments were documented through the first 60 d in laboratory-reared fish.

Regarding the early life history of Atlantic cod, it should be noted that I used eastern Atlantic cod which may be genetically different from the western Atlantic cod. The terms I used to define life history stages were based on the behavior of larvae/juveniles in the culture experiments. The pelagic phase may be related to the growth rate of a particular larva. The benthic phase was assigned to the period after utilization of yolk when the fish settle to a more benthic existence. It could be that the growth deflections were due to rate and size and not to the chronological age. The fish in the field analyses of Lough et al. (1989) may have been in the plankton longer because they demonstrated slower growth rates. Bolz and Lough (1983) did not find settling checks as they examined presettled fish. I was attempting to relate developmental events in the rearing situation with structures in the otolith. I believe that there is a relationship between ontogenetic events and distinct checks in the otolith (Fig. 2), and that by utilizing laboratory experiments one can follow what those life history events are and relate them to distinct marks in the otoliths. Checks may not be of identical time frames, but they may be produced by similar events. It is believed that the increments and checks have a reason for their formation and so are a record of a fish's life history.

The criticism on the use of fish length and otolith length is based on interpretations taken out of context. I state "The greater variability in otolith size and the smaller degree of shrinkage among older fish may diminish the ability and need to correct for shrinkage. Otolith size – body size inflections occur for intermediate sized larvae, and this would limit the ability to correct for shrinkage" (Radtke 1989, p. 1893). The statement that the otolith size did not change in proportion to growth rate should be qualified "within the present experiments" and that there may not have been enough differences in growth rate, especially at the young ages, to differentiate or show any variation in fish length – otolith data.

I will have to agree with the commentators that the point on fish length and otolith length may be a slight overstatement. The growth rate differences within the present experiments may not have been large enough to show the variation in otolith growth. In my experiments, data from the fish grown at 6, 8, and 10°C (e.g. Radtke 1989, fig. 8) were grouped together because there were no statistical differences ($P>0.05$) between the different groups in terms of growth rate and otolith size.

It is still my belief that the utilization of otoliths from shrunk larvae would decrease the variation due to shrinkage and size dependency. There is a multitude of literature to suggest that shrinkage is a major problem in ichthyoplankton surveys. Neilson and Campana go on to point out that the variation of otolith length to fish length is the rule rather than the exception. It should be kept in mind that in my study, otolith length and fish length were examined in early life history stages, and in the 15 reports cited, I found that many authors have successively discovered a fish length – otolith length relationship in the young stages. The literature cited can be narrated as follows.

(Templeman and Squires 1956): Otolith length is a fairly reliable indicator of fish length, but decreases as indicated with increasing age. Otolith mass may be a better gauge of fish size. Slow-growing fish produce heavier otoliths than fast-growing fish and there appears to be a sexual dimorphism. The citation by Templeman and Squires is taken out of context. Otolith size and, in this case, otolith length can provide a reliable estimate of fish length and this is supported by the finding of Templeman and Squires.

(Boehlert 1985): In adult fish (*Sebastes*), otolith thickness continues to increase while length remains constant. However, this is only in very old (>25 yr) fish. Statistically, it is possible to correlate otolith length with fork length. Otolith dimensions may be an excellent predictor of age.

(Krivobok and Shatunovskiy 1976): For all samples, a high correlation exists between otolith weight and fish length. However, when only fish of the same age class are considered, much variation is found. Otolith weight is a good indicator of fish size but not fish age, in this instance. Extra otolith growth occurs during the period of gonad maturation.

(Taubert and Coble 1977): Number of increments = number of days but is not related to fish length. A linear relationship was found between otolith length and fish length. However, the slopes are species and population specific.

(Marshall and Parker 1982): Otolith growth can be stopped by starvation and temperatures below 5°C. This will interrupt an otherwise reliable otolith diameter – body length relationship. "Regression lines and 95% prediction bands demonstrate a high correlation of fork length with otolith diameter for all groups (Fig. 6; Table 2)" (p. 545).

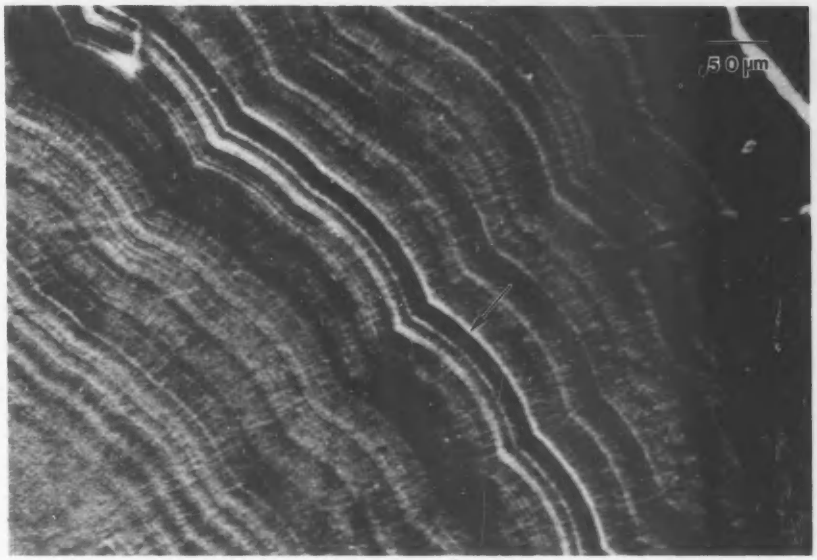

FIG. 2. Scanning electron micrograph of the check mark (arrow) of cod (*Gadus morhua*) which was related to the time of settlement or beginning of a more benthic existence in culture conditions.

There is evidence for an "engrained biological program" for increment formation.

(McGurk 1984): Increment deposition does vary in herring. The first normal increment is deposited at the time of fork absorption completion. The increment rate is proportional to the growth rate, and starvation retards both. Fish <20 mm have daily increment deposition if their growth rate >0.36 mm/d. Normally, average otolith deposition correlates with average growth rate. Fish size can be predicted from otolith diameter with a low degree of variance.

(Miller and Storck 1984): Successfully used otolith increments to age fish (young of the year juveniles). Stated "otolith radius and length of largemouth bass are highly correlated" (p. 574).

(Neilson et al. 1985): Successful application of otoliths to age salmon. Variation occurs when fish have been starved or heated (causing reduced food absorption efficiency) or when fish migrate from oceans to estuaries. 'The linear otolith length – fork length relationships also seemed adequate, as examination of residual plots of the unexplained variance gave no indication of curvilinearity" (p. 906).

(Penney and Evans 1985): In fishes of the genus *Sebastes*, otoliths are large, easily read, and otolith growth correlates well with body growth. Good evidence exists for daily periodicity. "Linear regression equations of length (millimetres) on sagittal radius (micrometers) ($TL = c \cdot R_T + d$) fit the data well in both years" (p. 1456).

(Reznick et al. 1989): Slow growers (guppies) have larger otoliths than fast growers. "This difference in relative otolith dimensions could aid in comparing growth rates among populations or could improve the use of otolith dimensions for estimating population age structures" (p. 108). These data support the use of otolith dimensions to correct for shrinkage problems.

(Secor and Dean 1989): Same results as Reznick et al. (1989) but for striped bass. The relationship between otolith dimensions and fish size is still correlated, but influenced by differences in growth.

(Mosegaard et al. 1989): Fish growth is uncoupled from otolith growth. Otolith growth is related to fish metabolism. Otolith mass is a better indicator of size and can be used to predict growth. Otolith mass is difficult to measure in larval stages.

(Rice et al. 1985): One increment is laid down per day under ideal conditions only. Otolith length is not well correlated with fish length. "For all short-term experiments, the relationship between total length and $\log_{10}$ (otolith diameter) was linear, but slopes were significantly different among experiments ($P<0.001$; Table 2)" (p. 536).

(Post and Prankevicius 1987): Increments are laid down daily but growth of otoliths is allometric to length rather than isometric.

(West and Larkin 1987): Otolith length and fork length of fish are only correlated sometimes. Starvation disrupts the correlation. The relationship varies between populations.

The criticism states that the above studies show that slow growing fish produced larger otoliths. This does not necessarily mean in length, it can be weight. There are papers that support the fish length – otolith length analyses.

There exists a functional relationship between the otolith radius and the length of fish. This relationship of proportionality between otolith and body growth holds for larvae as well as adults (Savoy and Crecco 1987; Messieh 1975; Radtke et al. 1985; Ralston 1976; Marshall and Parker 1982; Radtke et al. 1989). Recent evidence suggests that otolith dimensions can be used to age fish (Pawson 1990). These relationships make it possible to estimate the size of prerecruits.

It is surprising that Neilson and Campana substitute a fish length – otolith length relationship with a fish length – otolith dimension relationship. Otolith dimension can refer to a host of different measurements such as weight, volume, width, etc.

It should be kept in mind that an otolith is a three-dimensional object that has been formed in an aqueous solution. As

a consequence there is variability associated with deposition of a three dimensional object and with the physiology of the animal itself. I still conclude that otolith length can provide a reliable estimate of fish length, especially in the early life stages. This relationship may deteriorate as the fish age or growth rates increase substantially. The growth stanzas in the present experiments were unique to those experiments, while the data that are cited to rebuke this (Bolz and Lough 1983; Campana 1989) deal with wild populations and the associated sampling errors. The test for validity of the otolith diameter – fish length relationship is questionable. The use of Theilacker's (1980) algorithm, which was developed for shrinkage in anchovy, with cod lengths as provided by Bolz and Lough (1983) may not be an appropriate test. There is a large difference in otolith growth between species of the same genus let alone between genera (Boehlert 1985). But the switching of the utilization of fish length – otolith diameter to fish length – otolith dimension adds to the confusion of the comments.

I agree that there is a problem in the literature with the terminology, which is why I have tried to use the same terminology over the past 10 yr and always define my terms. I respect the authors objection, and suspect our differences could be in the agreement of "accepted" terminology. Until such time that there are rigorous chemical and physiological definitions or the mechanisms of physiology and biochemistry are known, the definitions of such terms will change.

Otolith function is reflected in the conservative nature of their growth. Otoliths are organs of equilibrium and hearing, and as such presumably must maintain precise configurations with respect to other parts of the fish's otic apparatus. This precise maintenance of size may not be necessary for other calcified tissues. It appears that otoliths are the first calcified structures to develop early in the lives of teleost fishes and that resorption does not occur once the otolith material is deposited. Incremental growth in otoliths occurs through differential deposition of calcium carbonate into a protein matrix over a 24-h period (Tanaka et al. 1981). The crystalline structure appears with the long axis of the crystals oriented perpendicular to the direction of growth. Individual carbonate crystals are meshed in a proteinaceous matrix band which apparently terminates crystal growth at each end (Dunkelberger et al. 1980). The crystalline structure usually found in otoliths is calcium carbonate in the aragonite configuration although vaterite is occasionally present (Campana 1983). The protein matrix has an abundance of acidic amino acids (Radtke 1984; Ross and Pote 1984), and structural components of this protein have been described by Watabe et al. (1982) and Mann et al. (1983). Little work has been done on the daily cycle of formation of the protein layer except for Tanaka et al. (1981). The rhythm of calcium deposition is probably regulated by endocrinological mechanisms (Simkiss 1974; Dacke 1979); however, parallels in calcium deposition between scale growth and otolith growth are difficult to identify. This is due to the fact that otoliths continue to grow under conditions of food deprivation (Marshall and Parker 1982; Campana 1983; Neilson and Geen 1982). Stress by exertion (Campana 1983), or by exposure to low pH (Geen et al. 1985), does not produce evidence of otolith resorption.

It is not feasible to compare and contrast within the confines of any article all written data available, and in the process some of the comparisons had to be eliminated sometimes due to referee suggestions. In particular the paper by Campana and Hurley (1989) was not available to the author prior to the acceptance of this paper, making comparisons between the data sets difficult.

In summary, I disagree with Neilson and Campana's overtone that the article (Radtke 1989) did not adhere strictly to the Journal's requirements for original information in terms of data pertaining to codfish otolith microstructure and their interpretation. I believe that the numerous workers in the field and the people interested in otolith microstructure and in cod will find the paper to be indeed one of the most thorough laboratory studies on otolith development.

All the data in the present report were collected from the experiments in Norway. It is true that I started research on cod in Canada in 1979–80. The results from this work have been published and the National Research Council of Canada acknowledged in four papers. The present study builds upon these earlier works — Richard L. Radtke, *School of Ocean and Earth Science and Technology, Hawaii Institute of Geophysics, University of Hawaii at Manoa, 2525 Correa Road, Honolulu, HI 96822, USA.* (JA648)

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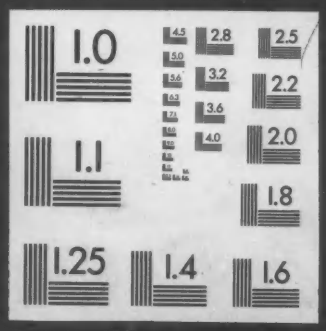
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